THE UNIVERSITY OF ALBERTA

MICROWAVE BIOEFFECTS IN THE DEVELOPING CHICK EMBRYO

by

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to my friend,

mentor, philosopher, and Father

James Douglas Fisher

ABSTRACT

Embryos of Gallus domesticus were incubated for 4 or 5 days and irradiated continuously over that period with 2450 MHz microwave radiation at a mean power density of 3.5 mW/cm^2. There was a significant difference in the cranial lengths, but not in the wet weights, of 4 day irradiated embryos when compared with 4 day control embryos. The magnitude and direction of the cranial length effect was temperature sensitive over the range of incubation temperatures studied (32°-$38^{\circ}C$). The manner in which cranial length varied with wet weight was also significantly influenced by microwave exposure. The above differences were not observed at 5 days of incubation.

The possibility that thermal artifacts may be responsible for many of the reported "non-thermal" effects of microwaves has been a source of controversy. Special attention was thus given in this study to the identification and control of thermal artifacts and to the influence they could have on the observed microwave bioeffects.

A possible "non-thermal" mechanism for the above temperature sensitive microwave bioeffect is proposed. This mechanism is discussed relative to previous work in the field.

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CHAPTER I

INTRODUCTION

Following Maxwell's theoretical description of electromagnetic radiation in 1865, Hertz demonstrated radiowaves experimentally in 1888. These discoveries set the stage for technological developments which made possible the generation of radiofrequency (RF) and microwave radiation at levels well above naturally occurring ones. The possibility of health hazards associated with exposure to non-ionizing electromagnetic fields, however, received little attention until 1960. Concomitantly the biological effects of RF and microwaves were not intensively investigated before 1960. In the welcoming address at the U.S. Air Force sponsored conference, "Biological Effects of Microwave Radiation" in 1960, Nelson stated:

> "Study of hazard from microwaves is still in a relatively early phase. It is at this stage that one asks the question, is there a risk, and if so, whence does it come, what are its characteristics, and what is the extent and the nature of the physiological effect? Beyond the interest and urgency arising from practical and humanitarian considerations, this is an exceedingly interesting scientific quest ....."

Although very loosely and arbitrarily defined, the microwave band may be taken as the frequency range from ∿100 to 300,000 MHz with wavelengths from 3 to 0.001 meters. The RF range, also poorly defined, includes frequencies from ∿0.03 to 100 MHz with wavelengths

of 10 to 0.003 kilometers. High intensity electromagnetic waves in either of these ranges will cause heat to be generated in biological tissues. Thus, it might be expected that at some level of radiation, biological tissues would suffer thermally. The American National Standards Institute (ANSI) has recommended, as a "protection guide", no more than "10 mW/cm^2 as averaged over any possible 0.1-hour period" for 10 to 100,000 MHz radiation (ANSI, 1966; 1974). This value (10 mW/cm^2) was arbitrarily chosen with "... consideration of tolerable rises in tissue temperature". The arbitrary nature of the recommended level is exemplified in the statement:

> "Radiation characterized by a power level ten-fold smaller will not result in any noticeable effect on mankind. Radiation levels which are ten-fold larger than recommended are certainly dangerous." (ANSI, 1966)

This standard is thus based exclusively on the thermal effects of microwaves on biological tissues. It must be remembered that biological tissues exposed to RF and microwaves will always exhibit a thermal response. Exposure to 10 mW/cm^2 or greater will, apparently, result in a physiologically "significant" temperature increase in tissues, constituting a "health hazard" (Tell, 1972; 1978).

The summed intensity of natural emissions, at frequencies below 300 GHz, from solar, meteorological and climatic sources is of the order of microwatts per square centimeter. Thus, the possibility of thermal effects did not cause concern until such man-made sources as radar and high power broadcasting and microwave systems came into intensive use.

Microwave heating is caused by the movement of dipolar molecules as they align themselves to the alternating electric field component of the radiation. Thus an increase in the intensity of the microwaves causes a corresponding increase in the movement and number of molecular reorientations and, therefore, in the heat generated within the tissue. The latter phenomenon is, however, limited by the dielectric properties of the material. At a point called the relaxation frequency, the dipoles can no longer effectively reorient themselves because of their size and the viscosity of their environment. Thus for a given dipole, heat generation as a result of molecular movement increases with frequency up to the relaxation frequency, after which it drops off. In considering biological materials exposed to the low end of the microwave range, water is the major molecular component contributing to the dielectric properties of biological tissues, with a broad band relaxation centered on a frequency of about 21 GHz. Since the frequency at which most microwave cooking ovens operate (2450 MHz) is below this relaxation band, microwave energy absorption by water molecules is also less and thus the depth of penetration in tissues is considerable. A frequency of 2450 MHz is thus well suited to the purpose of heating biological materials.

There are a number of reports suggesting that microwaves cause other biological effects more subtle than the general heating discussed above. One effect is the "auditory" perception of pulsed microwaves at mean power densities below 10 mW/cm^2 (Frey, 1961; 1975).

Another example of such an effect was reported by Tinney et al (1976): they found that bradycardia was induced in isolated frog hearts exposed to an 8 mW/cm^2, 2450 MHz field. When the field strength was increased to 40 mW/cm^2, which caused an increase in the temperature of the tissue, tachycardia was observed. Although there appears to be good evidence for these and other "non-thermal" effects, the nature and mechanism of occurrence have given rise to much debate and confusion. The difficulty is that until such effects are clearly and properly understood it cannot be determined which of these effects may be considered biologically "hazardous", and at what level.

Documentation of tissue heating and ensuing physiological effects resulting from exposure to radiowaves began in 1893. D'Arsonval reported hyperthermia in mice induced by 1 MHz radiation (1893; cited in Szymanowski and Hicks, 1935). Following this lead, Schereschewsky (1926) reported lethal elevations in the body temperatures of mice placed in an 8 - 135 MHz field. He noted that frequencies of 27 - 66 MHz caused the greatest number of fatalities. He attributed this to non-thermal "electromechanical vibrations in the cell" and suggested that the mice exposed to 27 - 66 MHz fields were more susceptable to pathological heating effects than were unexposed mice. Several investigators attempted to repeat these experiments with other biological systems: Kahler et al (1929) irradiated Paramecia with 10 - 75 MHz radiation, and Knudson et al (1931) exposed white rats to 9 - 12 MHz waves. While

deaths occurred in both experimental systems, neither investigation supported Schereschewsky's explanation. Instead, because the effects were independent of frequency, and solely dependent on intensity, they were attributed entirely to heating.

Christie and Loomis (1929) repeated Schereschewsky's experiments with mice and also found the effects to be frequency-independent. They attributed Schereschewsky's observations to artifacts resulting from his experimental method. The values given for power and frequency in the literature of this time are unreliable since the technology available to these early researchers did not permit very accurate measurements of these parameters. Schereschewsky had used only the current to the resonant circuit of the generator as an indication of field strength. It was pointed out that field strength may change with the frequency at a constant current because of dielectric changes in the medium as the frequency is varied. Christie and Loomis (1929) utilized instead a saline thermometer to assess field strength. Doubt was later cast on this method also, since, as mentioned above, heating is frequency-dependent to some extent. These difficulties were pointed out by Szymanowski and Hicks (1935), who suggested the use of both wavelength and current to the resonant circuit in calculating the voltage between the condenser plates. Although these early experiments were preliminary, and sometimes limited by the technology available, they did provide an indication of the multitude of technical difficulties that would have to be overcome before any

definitive statements could be made about the biological actions of radiowaves and microwaves.

Despite technical problems, several investigators realized the potential clinical applications of RF and microwave heating. Schereschewsky (1928) reported the total resorption of transplantable mouse and fowl sarcomas locally heated with 66-68 MHz radiation. Rabbits injected with Treponema pallidum failed to develop syphilitic lesions if treated with 10-14 MHz at 0.2 to 0.35 watts (Carpenter et al, 1930). RF heating or "transthermia" came into being at the turn of the century for the treatment of such ailments as rheumatism This has since become known as "diathermy" and includes microwave as well as RF heating. Diathermy is used today for muscle or joint ailments and, in conjunction with chemotherapy or X-rays, for the treatment of inoperable tumors.

In 1896, three years after his initial work on the thermal action of radiowaves, D'Arsonval reported that 0.2 MHz radiation significantly attenuated the strength of Diphtheria toxin. The same effect was later observed when the toxin was treated with 158 MHz radiation (Mellon et al, 1930). In Mellon's experiments, a thin film of toxin was cooled to 15^{o}C and exposed to 158 MHz radiation. There was a pronounced decrease in the toxicity of the preparation as compared with unirradiated toxin. Szymanowski and Hicks (1935) investigated the effects of 158 MHz radiation on the potency of Diphtheria, Tetanus and Botulinus toxins. They found that irradiation attenuated the toxicity of all three compounds. Although the

mechanism by which this occurred was unclear, thermal denaturation of the toxins was ruled out as the cause, since heating the toxins to the same degree with hot water failed to produce the same results.

Thus the controversy began. From the above experiments it was soon realized that the first step to its resolution was to determine the electrical properties and physical responses of biological materials exposed to the frequencies in question. Preliminary reviews by Schereschewsky (1933b) and by Szymanowski and Hicks (1935) stressed the need for accurate measurements of the dielectric constants and of the conductivities of biological tissues exposed to different frequencies of electromagnetic radiation. It was necessary to assess the degree to which these parameters affected in turn the absorption of radiowaves and microwaves. The impetus of research on the biological effects of microwaves up until about 1940 was primarily directed toward answering these questions. Richards and Loomis (1929) described changes in the dielectric losses of electrolyte solutions, and thus the heating, with changes in frequency and with changes in solute concentrations. Their results cast doubt on the use of the saline thermometer to measure field strength since the above parameters did, in fact, change with frequency.

Schereschewsky (1933a) had determined the rates of heating of various tissues exposed to radiation ranging in frequency from 64 to 300 MHz. He found, for example, that blood serum and heart muscle converted 300 MHz radiation into thermal energy at about the same rate. When the same tissues were irradiated with 64 MHz radiation,

the temperature of the heart tissue increased at a rate 1.7 times faster than that of the blood serum. Indeed, all the tissues tested had similar rates of heating at the higher frequencies. Thus it seemed justified to use higher frequencies, such as 915 MHz, for medical diathermy. The recognition that water plays a progressively larger part in radiowave energy absorption as the frequency is increased, and the relaxation frequencies of the larger dipoles are passed, may explain this phenomenon. The dielectric constants of brain, liver, muscle and kidney at 300 MHz, all approach the value of water alone, while fat, a tissue with low water content, was found to display a lower dielectric constant than these other tissues at 300 MHz (Clearly, 1970).

This prewar decade of research was concluded with the accurate determination of the relaxation frequencies for a variety of mono-peptides, dipeptides and tripeptides (Marcy and Wyman, 1941; Conner and Smyth, 1942; Conner *et al*, 1942). This gave some insight into how proteins might absorb radiation of different frequencies and thus how the biological effects of microwaves might vary with the tissue in question and with the frequency of the radiation.

These developments led to improved understanding of the quantitative and the qualitative aspects of molecular absorption of radiowaves and microwaves in tissues. Thus the thermal effects of microwaves could be more accurately characterized and the therapeutic potential of microwaves better understood. Equally important, however, was the value of these data in the formulation of hypotheses

predicting the many possible non-thermal effects. For example, it was recognized that a shift in the frequency of irradiation would quantitatively affect the absorption by various dipoles in a biological system, thus qualitatively changing the absorption mode of that system as a whole. The possibility of non-thermal, frequency-specific effects was thus suggested.

The construction of high power microwave generators for communications and radar was made possible by the technological advances associated with the Second World War. One of the results of these developments was a high incidence of pathological problems in some of the personnel working with these instruments. These effects were at first attributed to microwave-induced hyperthermia. Public concern about this new "environmental factor" prompted several large scale clinical investigations. Daily (1943) studied 45 men occupationally exposed to 10 mW/cm^2 radar. Hematological examinations and evaluation of the reproductive potential of these individuals showed these parameters to be within the normal range. Subjective complaints such as headaches were attributed to temporary heating as a result of occasional exposure to greater than 10 mW/cm^2 fields.

In 1954 a medical surveillance program was started on 335 airframe manufacturing personnel occupationally exposed to microwaves for up to 18 years (Barron and Baraff, 1958). The same parameters were measured as in Daily's studies, and the same conclusions were reached. Results such as these led to the establishment of a 10 mW/cm^2 maximum permissible human exposure level (MPEL) by United

States authorities. According to ANSI, continuous frontal exposure of a resting 70 kg man to 10 mW/cm^2, the MPEL, will result in a body temperature increase equivalent to that if the basal metabolic rate were doubled. This is considered, by ANSI, to be a "significant" temperature increase that may have pathological consequences.

The attitude taken by many researchers of this time was reflected in a statement made by G.M. Knauf, a colonel in the U.S. Air Force and renowned investigator in the microwave bioeffects field, at a conference in 1960: "..... we have not seen any research data which shake our faith in the validity of this arbitrary safe exposure level (10 mW/cm^2) .....". At this same conference however, Gunn et al (1960) suggested that changes in hormonal control of testicular function, indicated by a microwave-induced drop in Zn^{65} uptake by the rat prostate, were due to some, as yet undetermined, non-thermal mechanism. Frequency-dependent shifts in the electrophoretic pattern of human gamma globulins, resulting from exposure to non-thermogenic microwave fields were also reported at this conference (Bach et al, 1960).

Meanwhile the Soviet Union had established an MPEL of 0.01 mW/cm^2, based on the work of scientists in that country (GOST, 1976). Reports by Western scientists of apparent non-thermal effects prompted extensive reviews of the Eastern European and Soviet literature in this field, such as those by Dodge (1970) and by Healer (1970). These surveys revealed a surprisingly large volume of work that suggested non-thermal effects resulting from exposure to microwaves.

Thus, some Western scientists belatedly became aware of the 1957 review by Livshits, a Soviet researcher, of the early work in his country, on the biological effects of microwave radiation. This review revealed that Soviet researchers began, as did Western scientists, with the observation of microwave heating. One interesting difference, however, was that the Soviet workers saw that denervated organs heated at a significantly lower rate when exposed to UHF fields than did normally innervated organs. This led to the hypothesis that some microwave effects may be indirect ones, being mediated by the nervous system. From her work with chicken embryos, Van Ummerson (1961) has suggested, based on observations of microwave induced teratogenic effects, that non-thermal effects may influence the thermal effects resulting from high power exposure to microwaves. This view was also suggested by Phlomm's (1931; cited in Livshits, 1958) observation that the bradycardia induced in frogs, as a result of exposure to UHF fields, could be abolished with the administration of atropine, a parasympathetic blocking agent. This suggested that the observed bradycardic response was vagally mediated. This has been confirmed recently by western researchers: eg. Tinney et al (1976) also reported that microwave induced bradycardia could be abolished by atropine.

A second Soviet review by Livshits (1958) dealing with the neural effects of microwaves, referred to 91 papers, more than 40 of which were published before 1940. In the Western literature of this period, no comparable volume of information exists. Although

much of the Soviet work lacks such experimental details as specific frequency, exposure duration and intensity, the sheer numbers and consistency of the reports led some Western readers to take the claims seriously. One interesting hypothesis suggested by the study of the Soviet results is that transmission at myoneural junctions and excitation-contraction coupling are somehow altered by exposure to UHF fields. For example, small doses of UHF seem to shorten the latent period in the spinal reflexes of frogs, but at higher (thermal) levels the opposite effect is observed (Rozanova, 1939; cited in Livshits, 1958). This also lends credence to the theory that many non-thermal effects may be masked by the more obvious thermal effects at high irradiation levels.

The low Soviet MPEL was also based on clincial findings, again dealing mostly with purported nervous system effects. The extent of these findings is reflected in the fact that, in the Soviet Union microwave radiation sickness is a recognized pathological condition which, in extreme cases, requires hospitalization. The disease is said to be characterized by a variety of subjective symptoms such as insomnia, impotence, anxiety and amnesia. It is also defined by a number of more objective symptoms which include blood pressure changes and heart rate lability (Panov et al, 1966; cited in Dodge, 1970). Dodge (1970) stresses that the credibility of these findings again depends largely on the great volume of data together with its consistency. Experimental parameters such as microwave frequency and exposure duration, as well as environmental factors such as heat,

light, humidity, etc., are seldom included in the reports. Many Western researchers have voiced their impatience with this failure to report vital experimental parameters as well as with the subjective nature of many of the Soviet reports.

The state of confusion surrounding the arguments of the proponents and opponents of non-thermal effects probably increased, in no small measure, when Osipov (1965; cited in Michaelson and Dodge, 1971) suggested that many non-thermal effects may actually be microthermal in nature. As early as 1933, Schereschewsky (1933b) recognized that the size and shape of an organism relative to the direction of propagation and wavelength of the radiation affected the absorption of microwaves, in this case the heating of dead mice. It has been suggested from theoretical considerations, and from work with phantom models of humans, that the electrical and geometric inhomogeneities of the body (ie., the internal organs) could act as dielectric lenses in which low intensity, incident radiation could be focused so as to create a "hot spot" (Guy, 1974; Lin, 1975). Some of the auditory sensations such as "buzzing" and "clicking", reported by subjects in non-thermal intensity fields have been attributed to hot spots of this sort in the middle ear (Chou, 1977).

In spite of this confusion, an increasing degree of international and interdisciplinary cooperation in microwave research has recently evolved. The impetus of this research, over the past several years, has been directed toward elucidation of the possibility of non-thermal effects on electrically excitable tissue and on rapidly growing cells

and differentiating systems.

Extensive work on the teratological effects of microwaves on mealworm pupae (Carpenter and Livstone, 1971; Lindauer et al, 1974; Lui et al, 1975; Green et al, 1977; Pickard and Olson, 1977) chick embryos (Van Ummerson, 1961; Togotse, 1975) and mouse embryos (Beriznitskaya, 1973; 1977) have led several workers to conclude that aberrations in development, resulting from exposure to microwaves, cannot be totally explained in terms of thermal induction. This interpretation is, however, not shared by others who have found no reason to invoke non-thermal effects in experiments using quail embryos (McRee et al, 1975; McRee and Hamrick, 1977) or rat embryos (Rugh et al, 1975; Rugh and McManaway, 1977) as subjects. As noted earlier, Van Ummerson (1961) has suggested that microwaves inhibit differentiation in early chick embryos and that non-thermal effects may occur concomitantly and/or may act synergistically with thermal effects.

The possibility of non-thermal effects of microwaves on differentiation is also supported by experiments involving non-embryonic systems. Microwave radiation is the only known physical agent capable of inducing the premature transformation of lymphoblasts to lymphocytes (Stodolnik-Baranska, 1974). Several other investigators claim to have demonstrated other effects on hematopoiesis, such as changes in the amplitude and phase of the circadian rhythm of the bone marrow mitotic index in guinea pigs, after a 4 hour exposure to a 0.5 mW, 2.95 GHz field (Czerski et al, 1974). One clinical

study has indicated that long term exposure to low intensity microwave radiation may deleteriously affect spermatogenesis (Lacranjan et al, 1975). While the possibility cannot be ruled out that a testicular temperature increase may have been responsible for these findings, a recent study has shown that, in rats, microwave radiation will arrest spermatogenesis at a lower testicular temperature than if testicular function is arrested by heating with infra-red radiation or hot water (Fahim et al, 1975).

Thus no clear picture emerges in the literature concerning the effects of low intensity microwaves on growth. The quality and magnitude of reported effects appears to depend not only on the age and the species of the animal, but also on the microwave frequency and the mode of irradiation. Intermittent exposure to microwatt intensities of 880 MHz radiation had no effect on the growth rate of young chicks, while continuous exposure to the same frequency and intensity of microwaves depressed growth in both chicks and rats (Giarola and Krueger, 1974). Both 41 and 2450 MHz radiations increased the growth rates of chicks (Togotse, 1975), while in other studies 2375 MHz depressed chick growth (McAfee et al, 1973). McAfee (1973) also found, in contrast to Togotse's (1975) report, that 2450 MHz radiation had no effect on the growth rate of chicks. In some of the above cases, the involvement of non-thermal mechanisms has been proposed.

The above review is by no means complete. The selected examples do, however, serve to outline the controversy and varied complications

associated with investigating the effects, thermal and non-thermal, of microwave fields. This is best described in Justesen's (1977) paper entitled "Diathermy versus the microwaves and other radio-frequency radiations: A rose by another name is a cabbage". He begins the article by saying:

> "There are symptoms of schizophrenia in the body scientific with respect to the mixed but fervent attitudes of its constituents toward the potential perils of exposure to microwaves and other radio-frequency radiations. The Rose: exposure of human beings to fields of high power density is common, therapeutic and viewed with sanguinity in some sectors. The Cabbage: in other sectors the suspicion that even weak fields are present raises the pejorative finger of anguish and alarm."

A major problem in the interpretation of reported microwave bioeffects involves distinguishing between thermal and possible non-thermal effects. The experiments reported here explore the proposition that microwaves may athermally influence growth and differentiation in early chick embryos. The possibility that variation from optimal incubation temperature might modulate microwave effects is also investigated.

CHAPTER 2

MATERIALS AND METHODS

2.1 - Experimental Design

Most of the previous work concerned with the possible non-thermal effects of exposure to microwave radiation avoids discussion of the influence thermal artifacts may have on the results. However, because of the well known dependence of normal embryonic growth and development on optimal temperature, embryonic body temperature was included in this study as an experimental variable. Any temperature change, during or after irradiation with microwaves, was included in the final analysis of the data. The final body temperature of any single embryo thus included not only heat absorbed from the surrounding air but also any heat generated as a result of the absorption of microwave energy. In a trial run, however, there were no measurable temperature differences between control and irradiated embryos. Non-thermal mechanisms could therefore be proposed as the cause of any observed effects and the influence of body temperature on these effects could be determined.

The thermal induction problems encountered by others and the lack of effects, short of death, associated with short term, high intensity exposure regimes led instead to the use of chronic, low-level exposure in the present study.

The early (4-5 day) chick embryo was chosen as an experimental

system because of its small size and its biological suitability for the study of developmental effects. The chick embryo is a rapidly growing and differentiating system, readily obtained, easily incubated, and whose developmental pattern is well known. Nutritional self-containment precludes problems which might be associated with long-term, in vitro incubation, as of mammalian embryos. The possibility of indirect effects, via the mother, from the irradiation of mammalian embryos in utero, is also avoided, by use of the avian system.

Electromagnetic radiation in the visible range has been reported to affect the development of chick embryos (Lauber, 1975). Lauber has demonstrated that exposure of early chick embryos to visible radiation accelerates embryogenesis. The above study, therefore, set a precedent for investigating the influence of other non-ionizing radiation frequencies on the growth of chick embryos.

The dimensions of a chick embryo at this early stage of development are in all cases much less than ¼ wavelength, 2450 MHz having a free-space wavelength of 12.5 cm. The possibility of hot-spots developing within the embryo as a result of its own inhomogeneities is thus negligible.

Demorest (1978) has observed a high degree of correlation between cranial length, wet weight and the Hamilton - Hamburger stages of chick embryo development. Independently, either of these parameters could have served as an index of growth rate.

However, because of Demorest's finding of a consistently reliable relationship between wet weight and cranial length in normal chick embryo development, comparison of these parameters was also assessed in both irradiated and control embryos of this study.

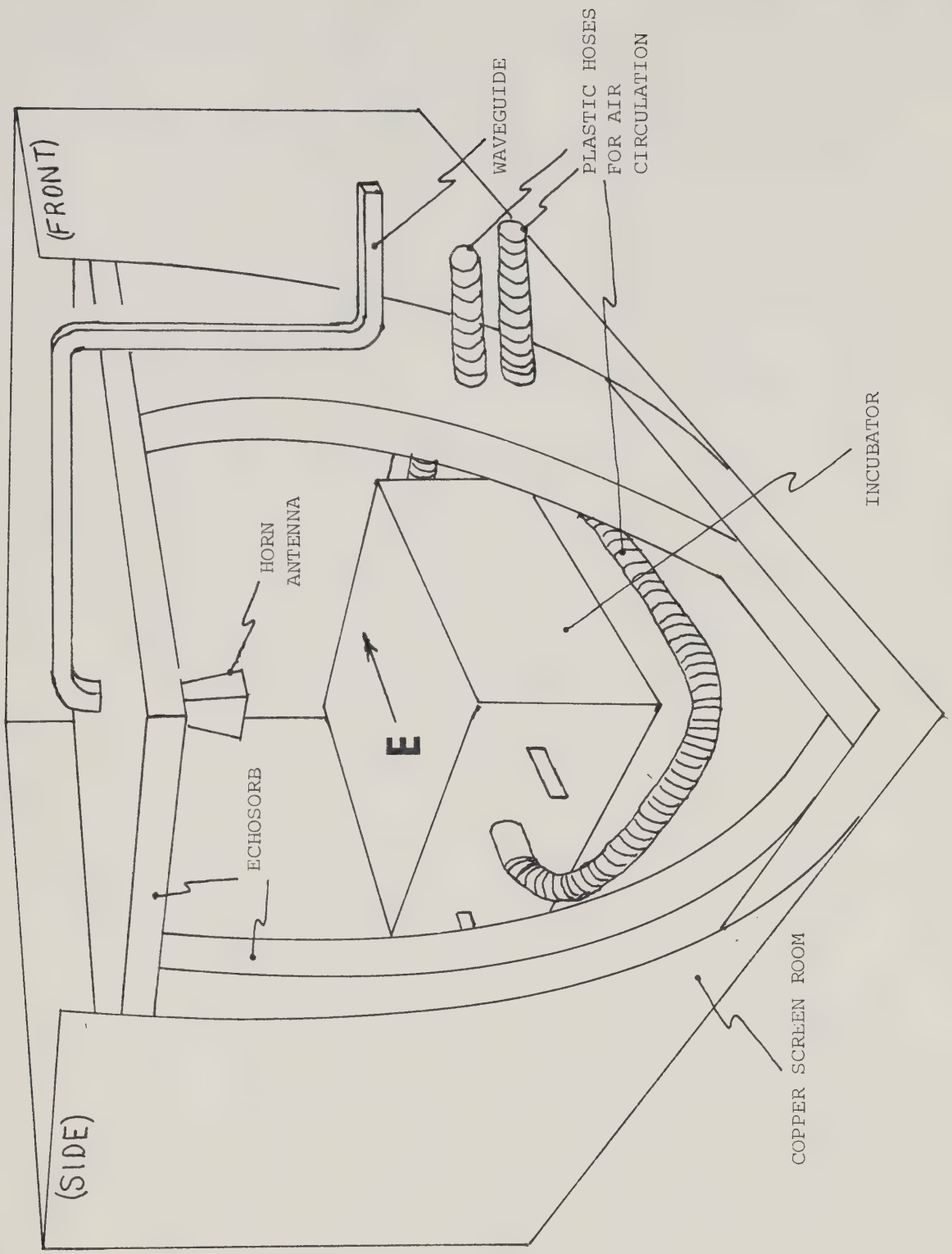
2.2 - The Incubation System

Commercially available incubation systems would have been unsuitable for this study of the biological effects of microwaves on chick embryos. The metal parts incorporated into commercially available incubators would have severely perturbed the electromagnetic field around the eggs. Therefore, the incubator had to be custom-designed and built of microwave-transparent materials.

The incubator and supporting apparatus are diagramatically displayed in Figures 1 through 6. The incubator itself (Figure 3) was located within an anechoic chamber (Figures 1 & 2) and connected, via nylon ribbed polyethylene hoses, to an external environmental control unit (Figure 6). Incubator construction details are shown in Figure 4, and a diagram of the egg turning mechanism in Figure 5. With this mechanism all of the eggs could be turned simultaneously through 60^{o} (30^{o} on either side of the vertical), along the direction of the E-field, without opening the incubator.

The air-conditioning system was a closed circuit. Air from the incubator was circulated through the conditioning system, by two fans placed in series, to be warmed and humidified and then returned to the incubator (Figure 6). A baffle at the intake port

Figure 1, A cut-away diagram of the anechoic chamber with the incubator in position. Shown are the relative positions of the screen room, the Echosorb enclosure, the incubator and the horn antenna. Refer to subsequent figures for more detail. The The large E in this and in all subsequent diagrams indicates the direction of polarization of the electric field component of the microwaves.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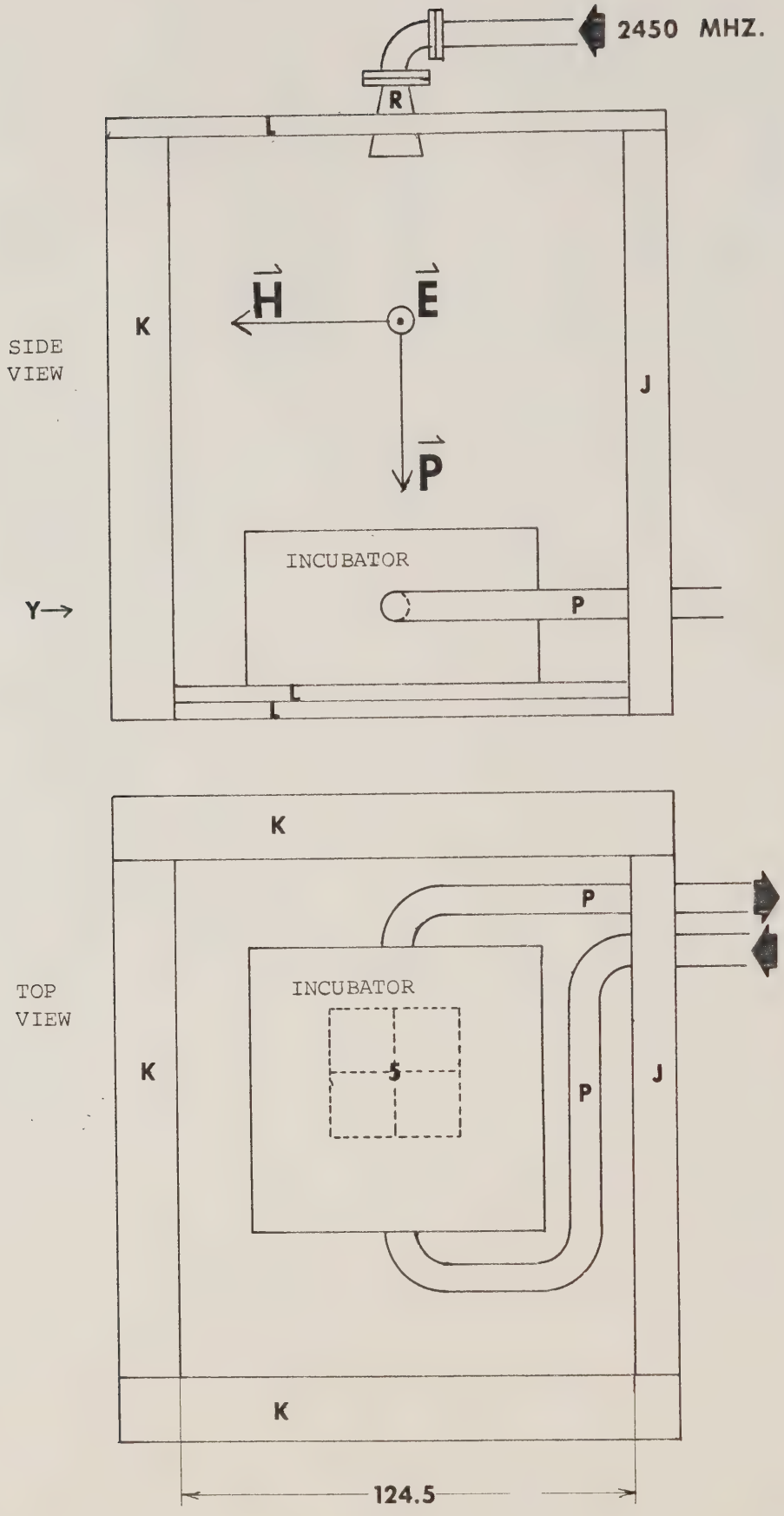
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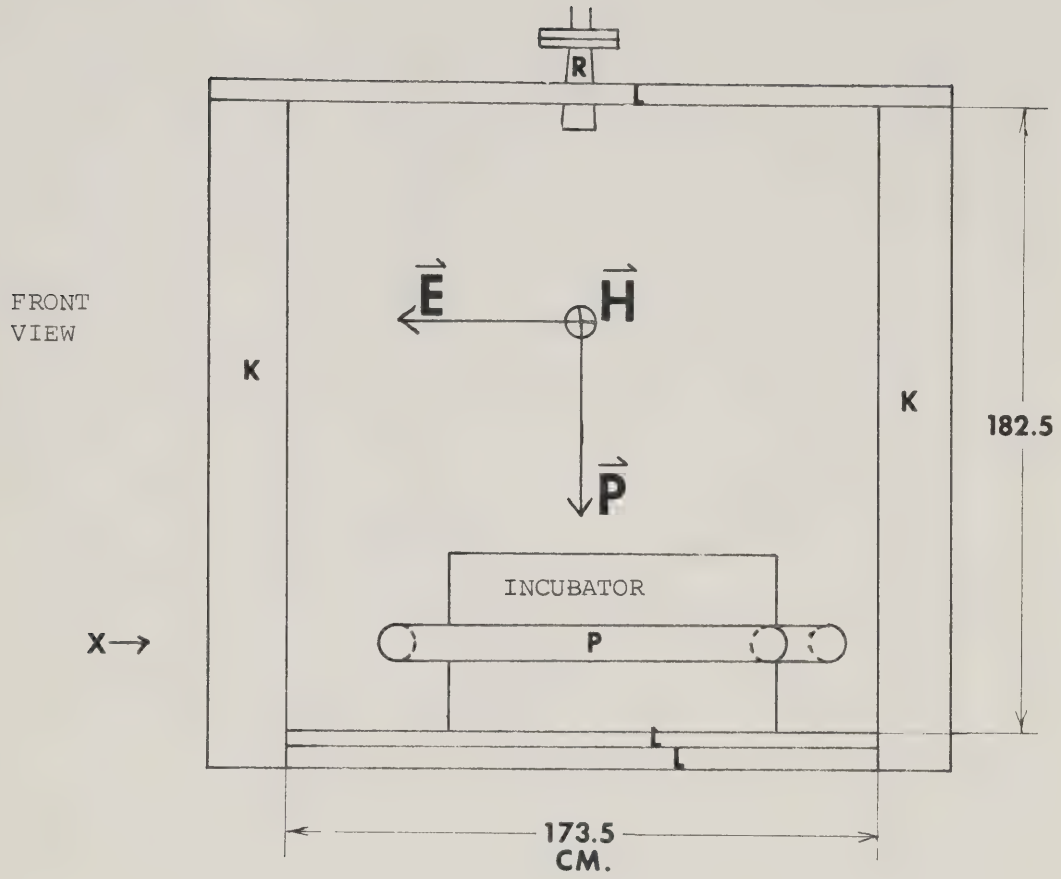
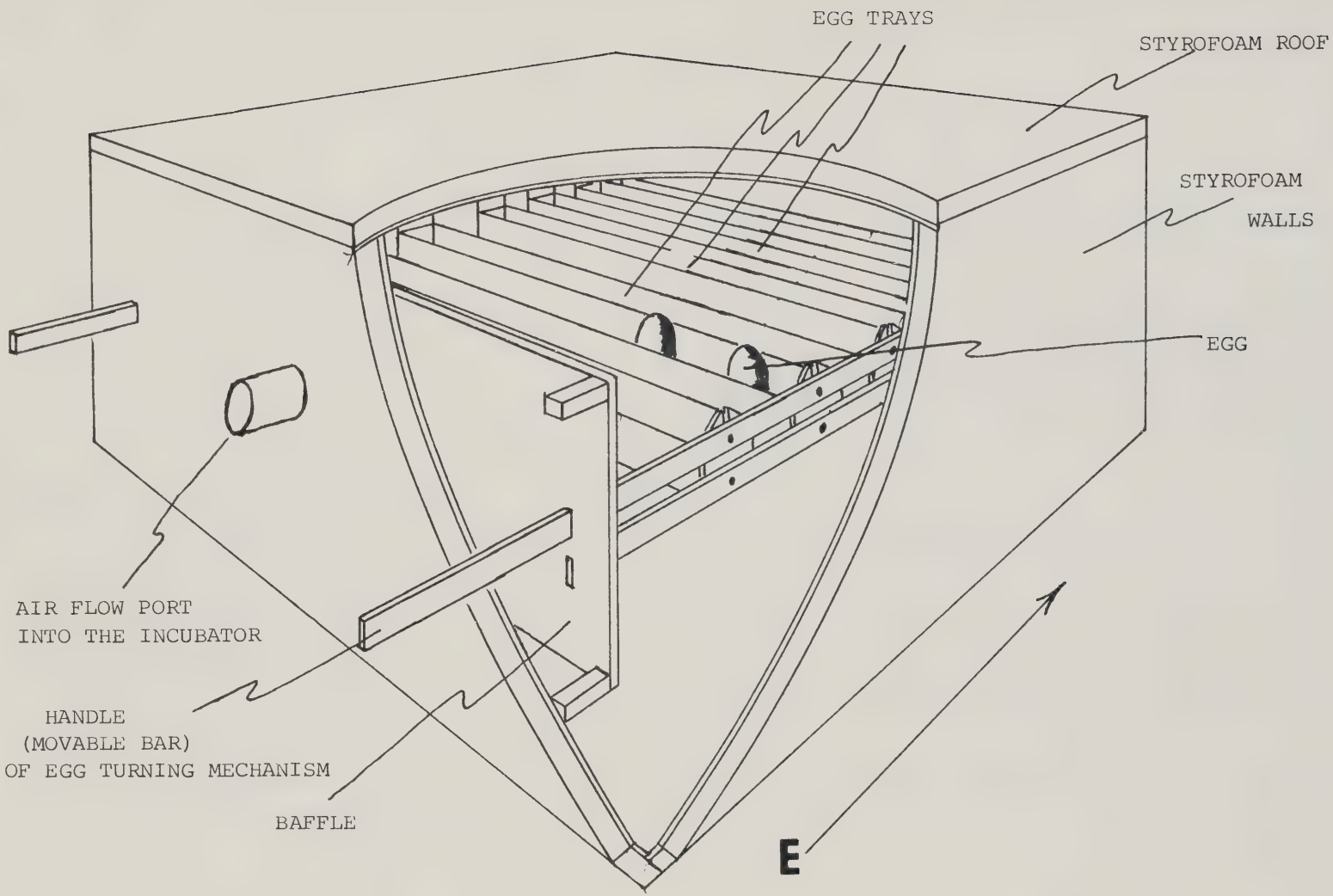
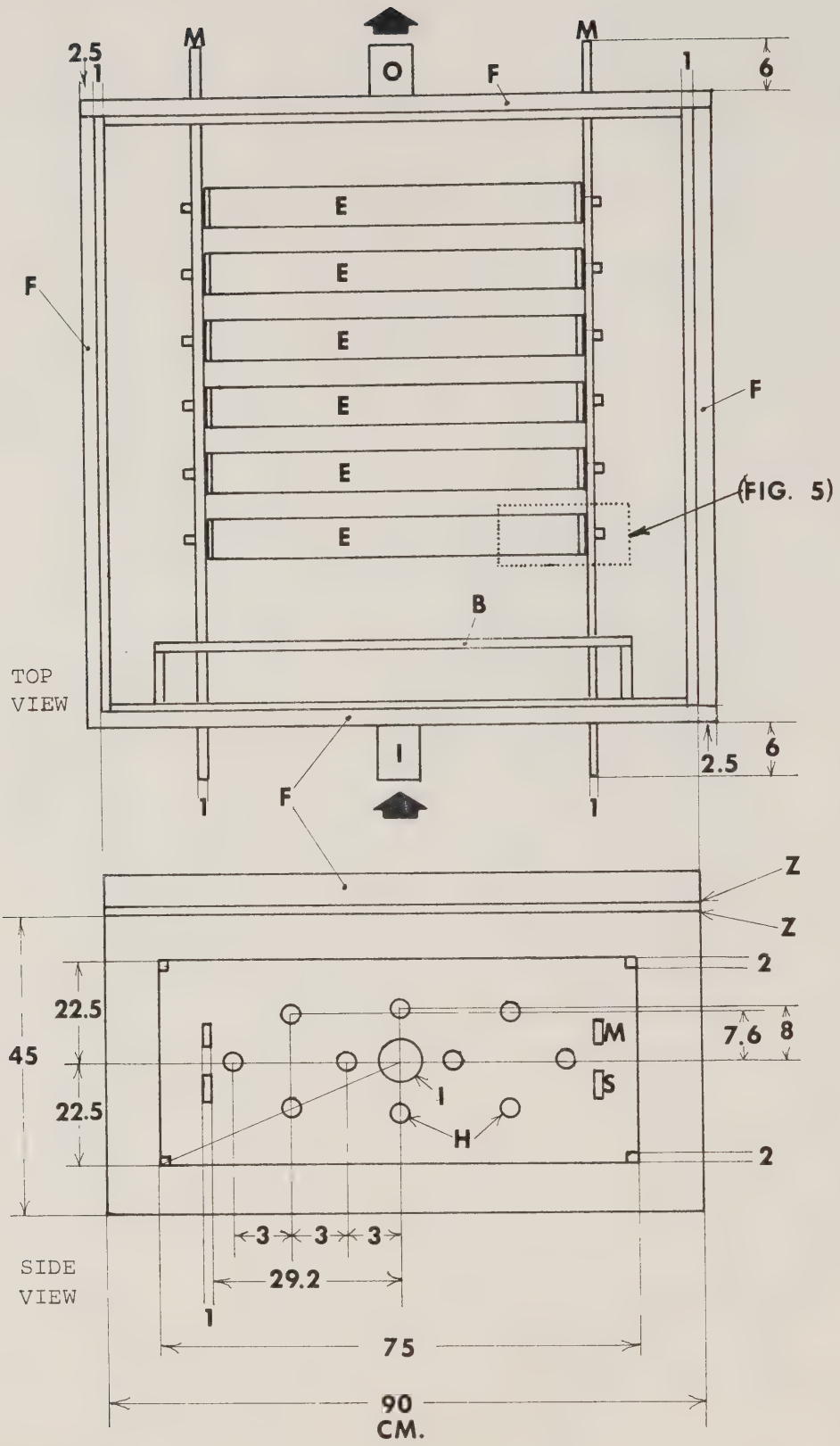


Figure 2, An expanded diagram of the anechoic chamber with the incubator in position and showing the relative position of the horn antenna (R). The screen room is not shown but surrounds the displayed structure with about 15 cm of air-space between it and the Echosorb. J, K and L represent blocks of Echosorb FR330 type I, ANW79 and FR330 type II, respectively. Air circulates between the incubator and the environmental control unit (Figure 6) via nylon ribbed polyethylene hoses (P). X and Y in the front and side views indicate the level of the axes of the matrix within the incubator (dashed lines) as shown in the top view. The center of the matrix is at "5" which is 168 cm below and on the axis of the horn. It is over this matrix that power density measurements were made as displayed in Figure 7 and Table 1. $\vec{E}$ and $\vec{H}$ are the electric and magnetic vector components, respectively, of the electromagnetic radiation. In the far field the power flow $\vec{P} = \vec{E} \times \vec{H}$ where $\vec{E}$, $\vec{H}$ and $\vec{P}$ are perpendicular to each other.

Figure 3. A cut-away diagram of the incubator. Refer to the expanded diagrams of the incubator (Figure 4) and of the egg turning mechanism (Figure 5) for more detail.


EGG TRAYS
STYROFOAM ROOF
STYROFOAM
WALLS
EGG
AIR FLOW PORT
INTO THE INCUBATOR
HANDLE
(MOVABLE BAR)
OF EGG TURNING MECHANISM
BAFFLE
E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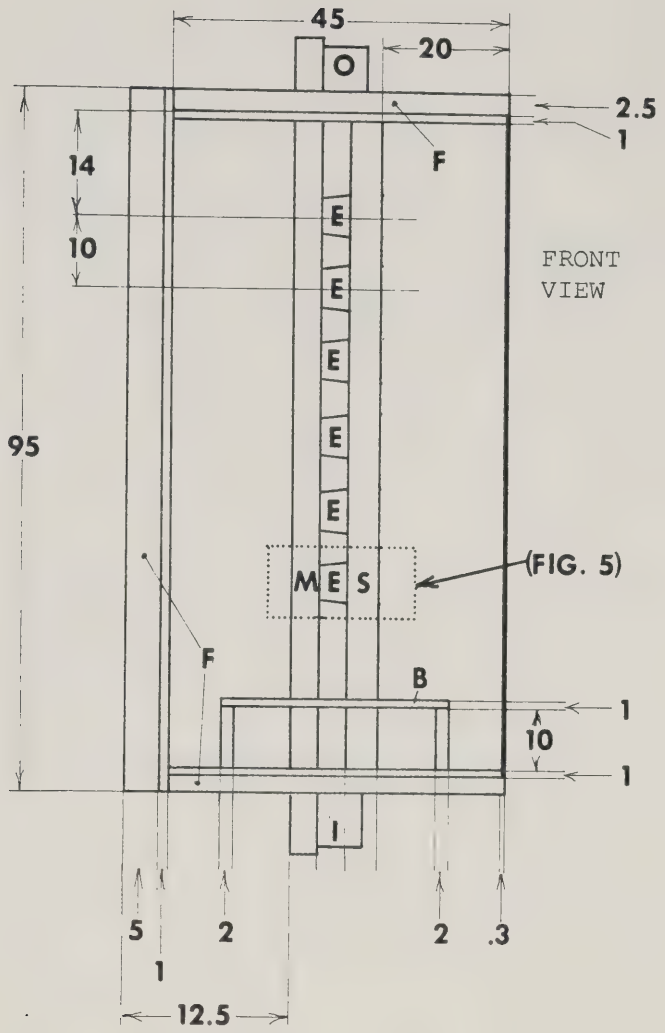
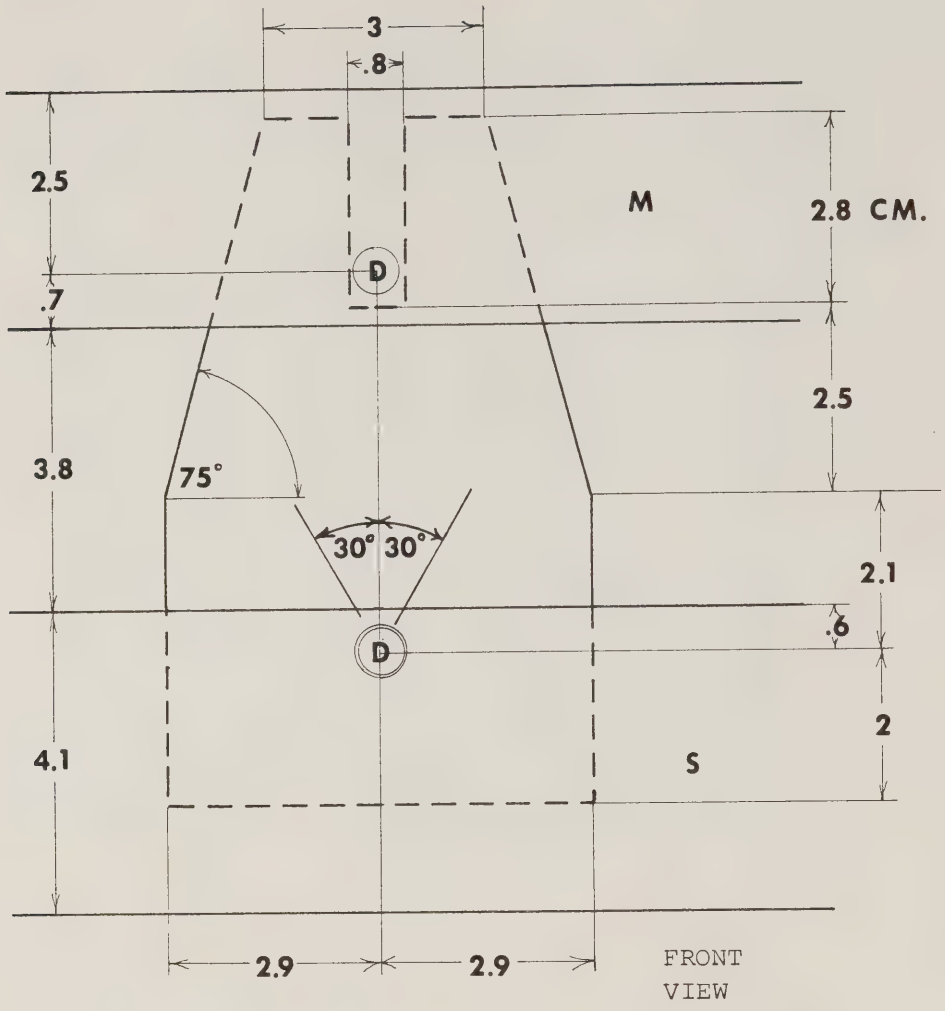


Figure 4, An expanded diagram of the incubator. Air is circulated between the incubator and the environmental control unit (Figure 6) via nylon ribbed polyethylene hoses connected to the intake port (I) and the outlet port (O) of the incubator. Air entering the incubator must pass through or around the baffle (B) perforated with 1" holes (H) providing for an even airflow over the eggs located within the egg trays (E). Egg tray detail is shown in Figure 5. M and S are the movable and stationary bars, respectively, of the egg turning mechanism. All parts are Perspex except for the styrofoam insulation (F) on the roof and walls, and the 4 mil. polyethylene sheets (Z) in the roof of the incubator. All dimensions are in cm.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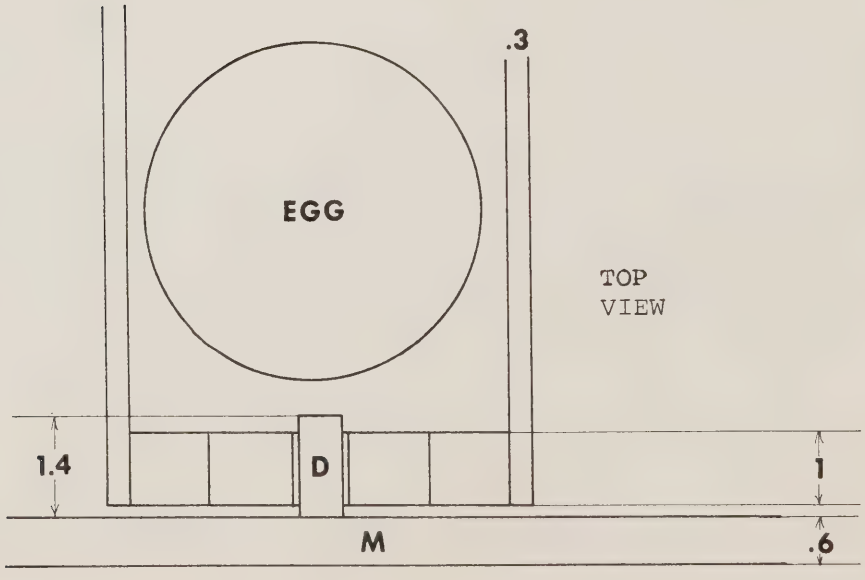
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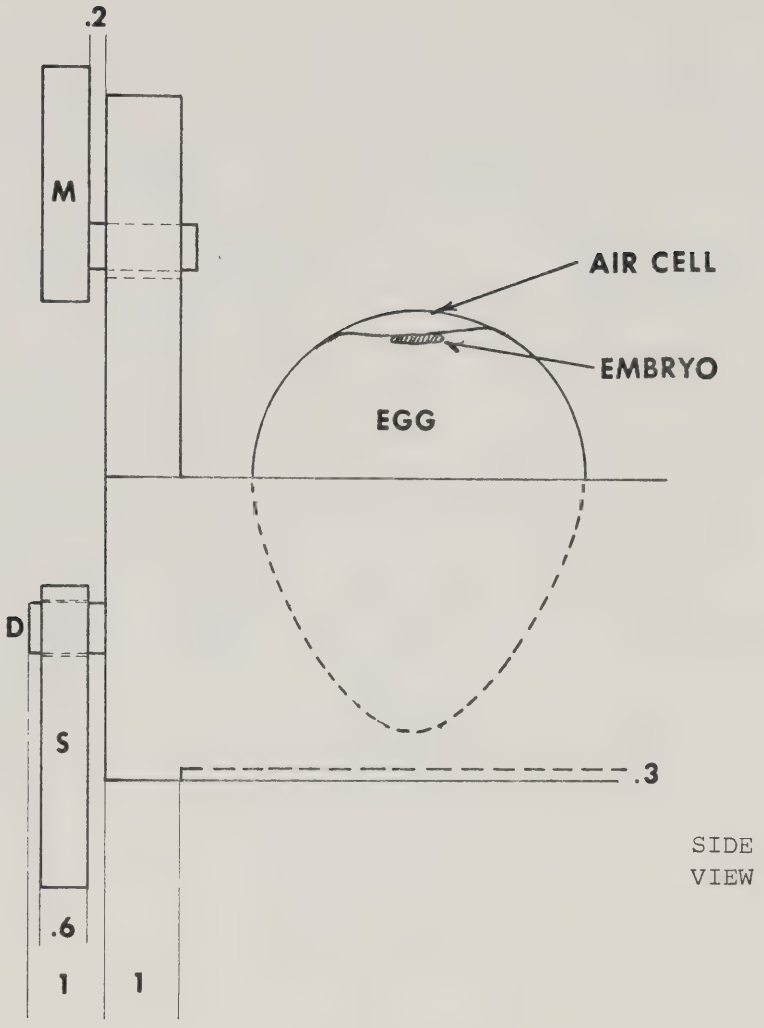


Figure 5. An expanded diagram of the end of one of the egg trays showing the egg turning mechanism. By pulling the movable bar (M) the egg tray, and the eggs within it, can be tilted 30° to either side from the vertical (see front view). S is the stationary bar which supports the egg trays and the eggs. D is ¼" perspex dowel. All dimensions are in cm.

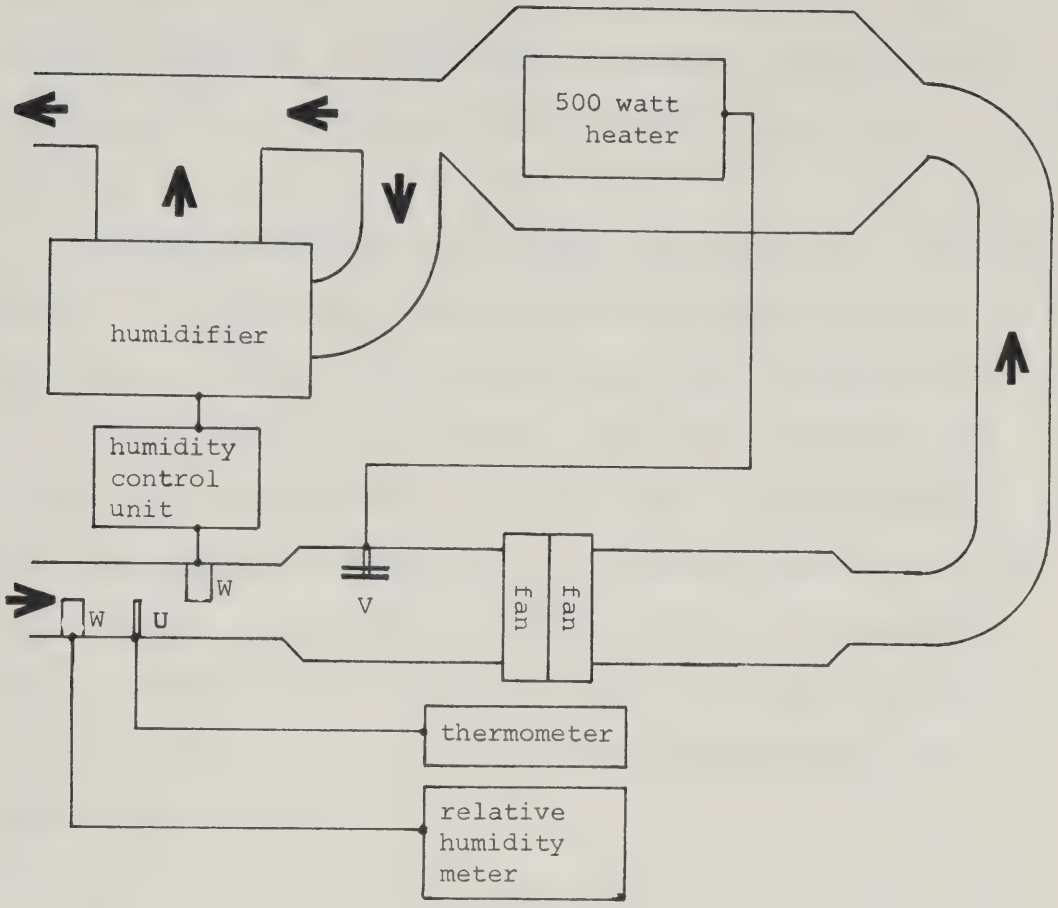


Figure 6. A schematic diagram of the environmental control unit. All connecting tubes are made of Perspex except that around the Edwin L. Wiegand (Pittsburg, Pa.) heater which is galvanized steel. The unit incorporates a Cool-Vapor humidifier (Hankscraft (Canada) Ltd., Toronto, Ont.), two Pamotor 4500C fans (Burlingame, Cal.) rated at 115 cu.ft./min. @, a Fluke 2100A digital thermometer (Mountainlake Terr., Wa.) and a Honeywell W611A relative humidity meter (Minneapolis, Minn.). The sensors included are: U - a Copper / Constantan microthermocouple, W - two Honeywell Q464A 1094 humidity sensors and V - a Brower's thermostatic wafer (Quincy, Ill.) activating a Honeywell WZ K Micro Switch. The arrows indicate the direction of airflow between the incubator (Figures 1, 2 and 3) and this unit.

of the incubator (Figures 3 & 4) was included in the incubator design in an attempt to insure uniform airflow over the eggs and thus minimize temperature and airflow gradients within the incubator.

Incubator temperature and humidity were regulated by negative feedback. A thermostatic wafer in the intake section of the environmental control unit (Figure 6) activated a microswitch connected in series with the heater in the outlet section of the control unit. The electronic humidity sensor and the humidifier were similarly placed (Figure 6). The humidity sensor activated, via an electronic control, a relay located within the power line of the humidifier when the humidity within the system dropped below a certain preset level. The relative humidity was thus maintained at 73±2%.

While the apparatus described satisfied the requirement for a microwave-transparent chamber, it was less than ideal in the temperature control which could be achieved. Temperature gradients over a 6°C range were observed over the array of eggs within the incubator. The effects of these gradients on embryogenesis are readily identified. Their influence on the growth of the embryos was included in the final analysis of the data as described in the 'Experimental Regime' section of this chapter. The air temperature at any one point within the incubator varied by ± 1.0°C as measured using thermocouples during a sham run.

2.3 - The Irradiation System

Electromagnetic radiation at 2450 MHz was produced with an American Microwave Inc. model 203 generator (Salt Lake City, Utah). The microwaves were transmitted via FXR (Woodside, N.Y.) and DeMornay-Bonardi (Pasadena, Cal.) S-band waveguide components and entered the anechoic chamber through a DeMornay-Bonardi DBL 15 db standard gain horn antenna. The aperture of the horn was located 168 cm above the top of the eggs. Forward power was measured within the waveguide using a Gerling Moore 4009 dual power meter (Palo Alto, Cal.). Before any biological experimentation began, the power density incident to the eggs, measured using a Narda Microline 8100 power density meter (Plainview, N.Y.), was plotted against the forward power readings. Any desired incident power could thus be set and maintanined by adjusting and monitoring the forward power.

The anechoic chamber was simply a copper screen room lined with Echosorb (Emmerson and Cumming, Inc., Canton, Mass.) Figures 1 and 2 show the relative positions of the incubator and the horn antenna within the anechoic chamber.

2.4 - The Electromagnetic Environment

The eggs were placed in the incubator in a 6 egg by 6 egg matrix, covering a square area 40 cm on a side, centered on and perpendicular to the axis of propagation of the 2450 MHz radiation. This area is represented in Figure 7 , which also shows the positions at which power density measurements were made at various forward powers (Table I).

Figure 7, Diagram representing the matrix over which power density measurements were taken at various forward powers. The relative position of the matrix within the incubator is shown in Figure 2. The power density measurements at the indicated positions are listed in Table 1 for various forward powers

P_f	matrix position								
watts	1	2	3	4	5	6	7	8	9
60	0.2	0.6	0.4	0.4	1.2	0.2	0.4	1.0	0.6
65	1.0	3.2	2.0	1.2	4.4	1.4	2.0	3.4	2.6
67	1.4	4.6	3.0	2.0	6.2	2.4	3.0	5.0	3.6
68	1.6	6.0	3.6	2.2	7.6	3.0	3.6	6.4	4.4
70	2.0	7.4	4.8	2.8	9.6	4.0	4.8	7.8	5.6
	power density mW/cm^2								

Table I, Power density measurements at the 9 matrix positions shown in Figure 7 for various forward powers. Position 5 is 168 cm directly below the horn.

From these preliminary measurements it was decided that a forward power of 67 watts would be most suitable to use in biological experiments to follow. At this power level the range of power densities at the 9 positions indicated in Figure 7 was from 1.4 to 6.2 mW/cm^2, with a mean power density of 3.47 mW/cm^2 (this is comparable to an average electric field intensity of 110 V/m).

The eggs, being located 168 cm away from the horn, were in the far field of the antenna. The location of the far field, which begins at 42 cm from the horn, is calculated from the equation:

$$r = \frac{2D^2}{\lambda}$$

where r is the near/far field boundary, D is the diagonal length of the aperture of the horn (D=16.32) and λ is the wavelength of the microwaves (12.5 cm). The design of the present study thus precluded the possibiity that the eggs would have been influenced by the induction

field of the radiator. The eggs were therefore assumed to be exposed to plane wave fronts whose intensities fell off according to the inverse square law.

A spectral analysis of the radiation was made using an HP 141 oscilloscope with HP 8555A spectral analyser modules (Hewlett-Packard Ltd., Mississauga, Ontario), in conjunction with an AEL model APN 101 A antenna (American Electronics Laboratories Inc., Lansdale, Pa.). The analysis revealed that the magnetron oscillated over the frequency range 2450 ± 5 MHz. Spurious outputs at 1200 and 2750 MHz accounted for less than 1% of the power.

2.5 - The Experimental Regime

Each experimental or control run used 36 eggs, weighing 50 to 60 grams each, from an outbred flock of Gallus domesticus maintained at the University of Alberta Farm at Edmonton, Alberta. Only eggs laid on the morning of the first day of each run were used. The incubator was turned on and allowed to prewarm for at least 24 hours before the beginning of the run. The eggs were placed small end down in the egg trays in the aforementioned 6 x 6 array (randomly distributed with respect to egg weight).

Incubation and, in the case of experimental runs, irradiation commenced at 1200 hours on the first day of each run. Irradiation was continuous for the 4 or 5 days of incubation except for 2 - 3 minutes, at 1200 hours, of each subsequent day of the run, during which the eggs were turned. The eggs were at all times 30° from the vertical along the direction of the E-field and were turned through

60° once every 24 hours. The eggs were in complete darkness during all incubation periods except for the short periods during which the eggs were turned.

Due to the spatial temperature gradients within the incubator, embryonic body temperatures varied between 32° and 38°C. The body temperature of any individual embryo varied by ± 0.4°C with time. From a series of sham runs in which the temperatures of all 36 embryos were monitored with implanted microthermocouples it was found that the temperatures of all the embryos could be reliably estimated, with an accuracy of ± 0.4°C, from the terminal temperature measurements of only 6 embryos. This indirect method of assessing temperature became essential since thermocouples could not be placed in the microwave field. Body temperature measurements could only be made after opening the incubator at the end of the run. The rapid rates of cooling of the eggs, ∿ 1°C/min., after opening the incubator were such that the temperatures of no more than 6 embryos could be quickly and accurately measured.

Thus, at the end of each run, the embryonic body temperatures of a consistent set of 6 of the 36 eggs were rapidly measured using a Fluke 2100 A digital thermometer (John Fluke Mfg. Co., Inc., Mountainlake Terr., Wa.) and a copper-constantan microthermocouple. The thermocouple was thrust through the air cell and against the body of the embryo via a hole made in the shell at the blunt end of the egg.

Subsequent to recording the temperatures each embryo was excised and the extraembryonic membranes removed. The embryos were weighed wet on a Federal Pacific Co. model LG precision torsion balance

(Northboro, Mass.). A right side view photograph was taken with a Nikon F camera through a Medical-Nikkor Auto lens (Nippon Kogaku K.K., Tokyo, Japan), immediately after weighing. Cranial measurements were taken as indicated in Figure 8, from photographic enlargements of the embryos.

Exactly the same procedure was used for control embryos as for experimental embryos, with the exception that the microwave generator was not turned on for the control runs.

2.6 - Data Analysis

Log_{10} transformations of both the wet weights and the cranial lengths revealed a linear relationship between these parameters and the embryonic body temperature. This facilitated subsequent analysis which was applied exclusively to the log_{10} transformations.

Since embryonic temperatures were estimated from the measured temperatures of a consistent set of 6 eggs, and the temperature of any single egg varied by ± 0.4°C with time, these temperature measurements had to be considered as being "subject to error" when doing the statistical analysis. This precluded the use of the commonly employed type I regression, which assumes no error in the parameter. Bartlett's type II regression (Sokal and Rholf, 1969) was therefore applied. Since this type of regression analysis does not include any index of the linearity of the data, correlation coefficients have been provided.

Type II regression analysis as well as correlation analysis were applied to wet weight and to cranial length data plotted against the final body temperature of irradiated and of control

Figure 8, Diagram of a chick embryo of approximately 4 days incubation age, (from Patten, 1957) showing the dimension that was measured as cranial length (C). Measurements were taken from enlarged photographs which had been made of freshly excised embryos.

embryos incubated for 4 or for 5 days. Regression and correlation analysis were also applied to the cranial lengths plotted against the wet weights of the control and of the irradiated embryos after 4 and 5 days of incubation.

Comparison of the data from irradiated and control embryos was made using a t-test for the difference between regression coefficients and a t-test for homogeneity among correlation coefficients. These tests were applied to the wet weight vs temperature data, the cranial length vs temperature data and the cranial length vs wet weight data from control and from irradiated embryos incubated for 4 or for 5 days.

2.7 - Absorption of Microwave Energy

In the case of organisms exposed to non-ionizing radiation as as environmental factor, it would appear to be necessary to describe qualitatively (frequency, polarization and field pattern) and quantitatively (incident power density) the electromagnetic environment to which the organism is subject. This has been detailed in a previous section, for these experiments. It is also essential to know how much energy is being absorbed by an organism exposed to microwave radiation, in absolute terms (dosimetry) and relative to the incident power (densitometry).

For microwaves of thermal intensity this determination is easily and accurately made by thermographic methods. At non-thermal intensities, as in this case, such methods are not applicable.

Extrapolation to low intensity absorption from the heating rates of organisms exposed to higher intensities is one way of

estimating absorption at lower levels of irradiation. In theoretical terms, however, this method is undesirable. The reason is that at different temperatures the heating characteristics of a given material change as a result of the effect heat has on the dielectric properties of that material, which in turn determines the absorption of microwave energy. The complex nature of a biological system precludes the use of a simple mathematical term to compensate for these changes when doing such an extrapolation. The method used in this study and described below, although not without difficulties, would appear to be decidedly more accurate than the extrapolation method described above. The apparatus described in Figure 9 was used to determine the absorption, by an egg, of 2450 MHz radiation with a forward power of 10 mW. The egg, which weighed 54.8 gm, was placed within the waveguide system and the transmitted and reflected power measured. These measurements were compared to those made with the egg absent. The egg was then cleared by blowing the contents out via holes made at both ends of the egg. The empty shell was then filled with water and the holes sealed with paraffin. The same measurements were made using the water-filled eggshell as with the intact egg.

This system was also used to determine the degree of perturbation introduced by the egg trays, as well as the amount of power reflected by the floor of the incubator. This was achieved by placing a section of the egg tray, or a section of 3/8" plexiglass, at the appropriate angle within the sample chamber and measuring the

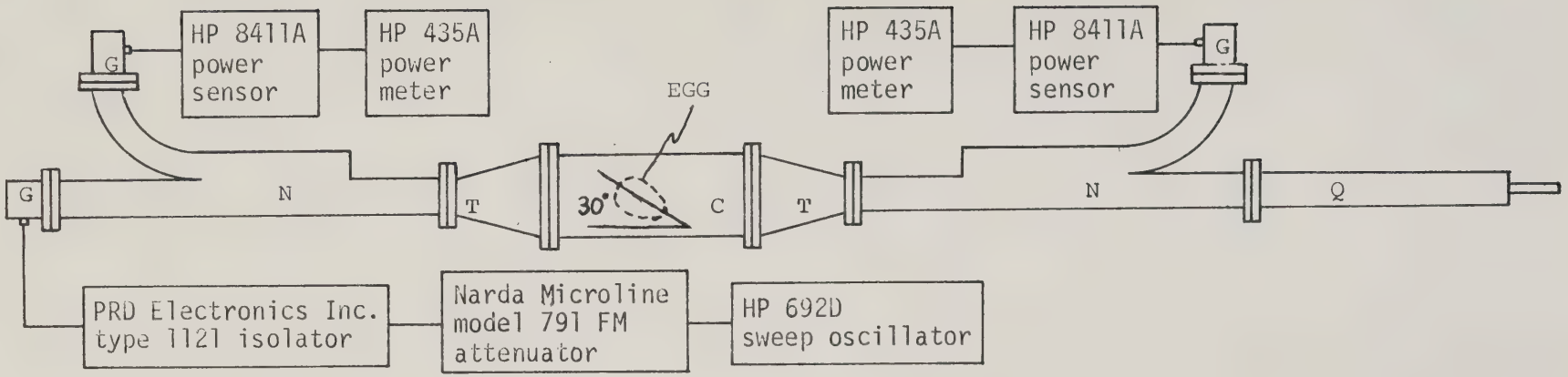


Figure 9. Waveguide apparatus used to determine the approximate levels of absorption and reflection of 2450 MHz radiation by a sample egg. For results of these determinations see Table 2. The sample egg was placed in the sample chamber (C) with its long axis 30° from the direction of microwave radiation. Components include: G - Microlab/FXR S601B adapters (Livingston, N.J.), N - Systron Donner DBL-675-10 directional couplers (Concord, Cal.), Q - HP S914A moving load, T - 17 cm long tapered waveguide sections betweem the standard size 2450 MHz wave guide and the sample chamber, C - a waveguide sample chamber with internal dimensions 10.9 cm x 5.45 cm x 30 cm. The output frequency of the sweep oscillator was fixed at 2450 MHz using a Beckman 6148 eput and timer (Richmond, Cal.) in conjunction with a Beckman 609 heterodyne. All HP devices were obtained through Hewlett—Packard (Canada) Ltd., Mississauga, Ont.; the isolator through PRD Electronics Ltd., Westbury, N.Y. and the attenuator from the Narda Microwave Corp., Plainview, N.Y.

transmitted power, the reflected power and the voltage standing wave ratio, all of which were compared with the same measurements taken when the system was empty.

CHAPTER 3

RESULTS

3.1 - Power Absorption by the Eggs and Field Perturbation

Prior to evaluating the effect of any environmental factor it is necessary to describe that factor in both qualitative and quantitative terms. Qualitatively, the factor being studied here for its biological effects is microwave radiation at a frequency of 2450 MHz. The pattern of the field to which the embryos are exposed may be perturbed by objects within that field, such as the eggs and the parts of the incubator. Before one can accurately describe the field to which the embryos are subject, one must first determine the influence of the eggs and the incubator on the field.

Table II lists the results of preliminary experiments designed to give an indication of how and to what degree different parts of the incubator and the eggs affect the microwave field to which the embryos are exposed.

These experiments were conducted using the apparatus described in Figure 9. A 5 cm long section of egg tray (Figs. 4 & 5) placed within the sample chamber 30° from the axis of propagation, the same orientation as in the biological experiments, absorbed 2.4% of the forward power (10 mW) and reflected 0.7% of it. A 3/8 inch 10.5 x 5.3 cm perspex plate placed perpendicular to the axis of propagation served as a model of the floor of the incubator. It

absorbed 5.3% and reflected 1.8% of the forward power. The voltage standing wave ratios derived from the per cent reflected power (Saad, 1963) and recorded in Table II, indicate that field perturbation around the eggs resulting from the interactions of the microwaves with the egg trays and the floor of the incubator is probably negligible.

	P_t	P_r	P_a	$P_r\%$	VSWR
empty	10.0	0.01	0.00	0.1	1.065
egg tray section	9.7	0.07	0.24	0.7	1.183
3/8 inch plate	9.3	0.18	0.53	1.8	1.310
egg + egg tray section	2.8	2.70	4.41	27.0	3.2
eggshell filled with water + egg tray section	2.0	2.90	5.11	29.0	3.3

Table II, Results of experiments to determine the effects of the components listed in the first column on a microwave field within a waveguide and to determine the amount of microwave power absorbed by the egg and embryo. The apparatus used is displayed in Figure 9. P_t, P_r and P_a are the power, in mW, transmitted, reflected and absorbed, respectively. $P_r\%$ is that per cent of the forward power that is reflected. VSWR is the voltage standing wave ratio determined from $P_r\%$ (when $P_r\% = 0$; VSWR = 1). Forward power in all cases was 10 mW.

Radiation reflected from the roof of the incubator towards the eggs would severely perturb the field around the eggs. The roof of the incubator is assumed, however, to be completely transparent to microwaves because its component parts are either very thin relative to the wavelength of the microwaves (the polythylene

sheets) or of very low density (the styrofoam) (see Fig. 4). Thus any radiation reflected by the eggs in the direction of the roof of the incubator probably passes through the roof to be absorbed by the Echosorb ceiling of the anechoic chamber. It is assumed that the eggs are exposed to almost plane wave fronts insignificantly perturbed by the incubator.

Although the incident power density to the eggs has been recorded it is also desirable to determine how much of the incident power is being absorbed by the egg and the embryo. For reasons described in the previous chapter, the easiest and most accurate way of achieving this is to determine how much of the forward power <u>is not</u> being absorbed by the egg.

The apparatus described in Figure 9 was used to determine the amount of the forward power reflected and absorbed by the sample egg. Table II lists the results of these determinations. An egg weighing 54.8 grams placed in the sample chamber with its long axis 30^{o} from the direction of propagation of the microwaves simulated the orientation of an egg in the incubator, relative to the field. The egg thus placed absorbed 4.4 mW of the 10 mW forward power and reflected 2.7 mW. Measurements of the reflected and transmitted power when the sample chamber was empty (Table II) indicate that the egg is responsible for nearly all of the reflected or absorbed power when the egg is in the chamber. The sum of the reflected and absorbed power, 7.1 mW, should therefore, be equal to

the power incident to the egg. The egg's cross-sectional area, perpendicular to the direction of propagation, was about 15 cm^2. Thus the incident power density was approximately 0.47 mW/cm^2. When the shell of the sample egg was filled with water and placed within the microwave field it absorbed and reflected almost the same amount of power as did the intact egg (Table II). Water is an efficient attenuator of microwave radiation, about 90% of the power being absorbed in the first cm penetrated at 2450 MHz. Any of the incident microwave radiation that is not reflected by the egg may therefore be assumed to be absorbed by it, with a negligible amount being transmitted through the egg. The sum of the power absorbed by and reflected by the egg is thus a valid estimate of the total power incident to the egg.

An egg oriented within a microwave field as described above thus reflects about 40% of, and absorbs about 60% of the power incident to it. The mean incident power density used in the present biological experiments was 3.5 mW/cm^2 over a range of 1.4 to 6.2 mW/cm^2. The total mean incident power to individual eggs would, therefore, be about 53 mW over a range of 21 mW to 93 mW assuming a 15 cm^2 mean cross-sectional area perpendicular to the axis of propagation of the microwaves. An "average" 55 gram egg exposed to a field of the aforementioned intensity would absorb power at a mean rate of about 0.6 mW/g (0.51 cal/g/hr) over a range from 0.4 to 1.0 mW/g (0.35 to 0.86 cal/g/hr).

From the above, the mean absorption rate of a 4 day embryo weighing approximately 30 mg would be 0.02 mW. This value, however,

must be an underestimate since most of the power that will be absorbed is deposited in the first cm of the egg (ie. where the embryo is). At 3.5 mW/cm^2 incident power, 2.1 mW/cm^2 (60%) would pass into the egg. The power absorption in the first cm of the egg substance, including the embryo, would thus be slightly less than 2 mW/g (1.72 cal/g/hr) which for a 30 mg embryo means an absorption rate of 0.06 mW (0.05 cal/hr). The embryos are thus absorbing microwave energy at a rate between 0.6 and 2.0 mW/g but probably closer to 2.0 mW/g.

3.2 - Developmental Effects

The incidence of deaths and abnormalities among irradiated embryos was not significantly different from that found in the control groups (Table III).

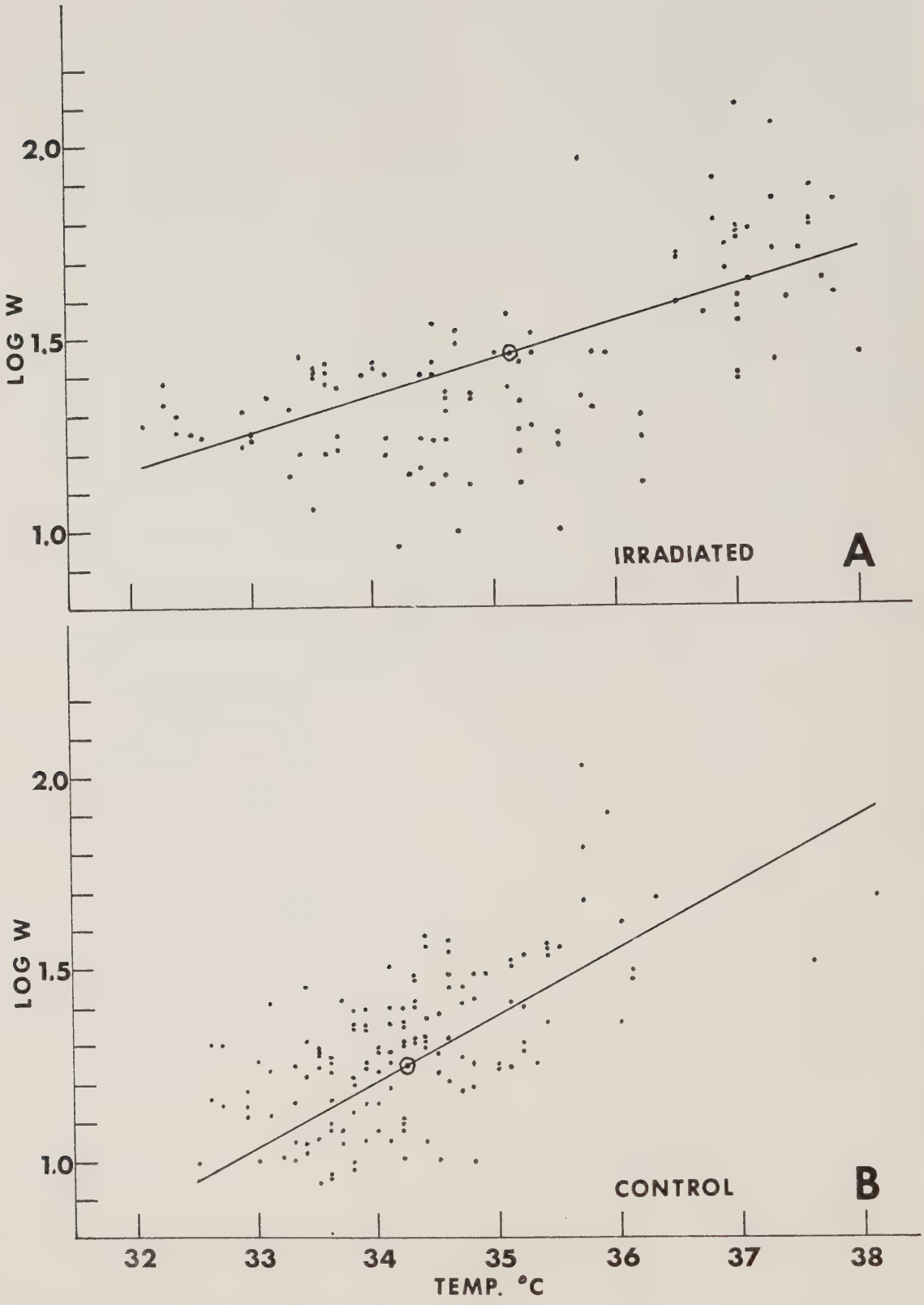
days incubated	4	4	5	5
exposure regime	irrad.	sham	irrad.	sham
n	100	65	103	88
% normal	90.1	89.3	81.6	89.8
% abnormal	7.0	10.7	14.6	9.1
% dead	2.9	0	3.8	1.1

Table III, Incidence of death and abnormality observed in the experiments; n excludes those embryos accidently ruined in manipulating them during excision and those eggs which were infertile; % dead includes those embryos whose development was arrested regardless of probable cause. Abnormal embryos were those which were still alive but had suffered relatively gross developmental deformations (exencephaly, two-tails, small eyes, malformed heart-tube, etc).

While non of the early deaths or abnormalities observed could be attributed to the microwaves, the incidence of each of the above was greater than about 10% in all cases. It must be remembered, however, that all the eggs were incubated at sub-optimal temperatures. Dead or abnormal embryos were not included in the regression or correlation analysis.

The wet weights of the irradiated embryos were in all cases insignificantly different from those of the control embryos incubated for the same duration at the same temperature (Figures 10 & 13, Tables IV & V). The cranial lengths of irradiated embryos incubated for 4 days were found to vary with temperature and with wet weight in a significantly different manner than did those of the control embryos (Figures 11 & 12, Table IV). These responses were no longer significantly apparent in embryos incubated and irradiated for 5 days (Figures 14 & 15, Table V). The cranial lengths of 4 day irradiated embryos with body temperatures above 35°C were less both in absolute terms and relative to wet weight, than those of the 4 day control embryos incubated at the same temperature. In the case of embryos with body temperatures below about 35°C the reverse of the above is observed. Parts A and B of Figures 10 to 15 include scattergrams of the transformed data and the regression lines calculated from that data. Part C of these figures shows only the regression lines from parts A and B to facilitate the visual comparison of the data from irradiated versus control embryos.

The correlation of the data used to calculate each regression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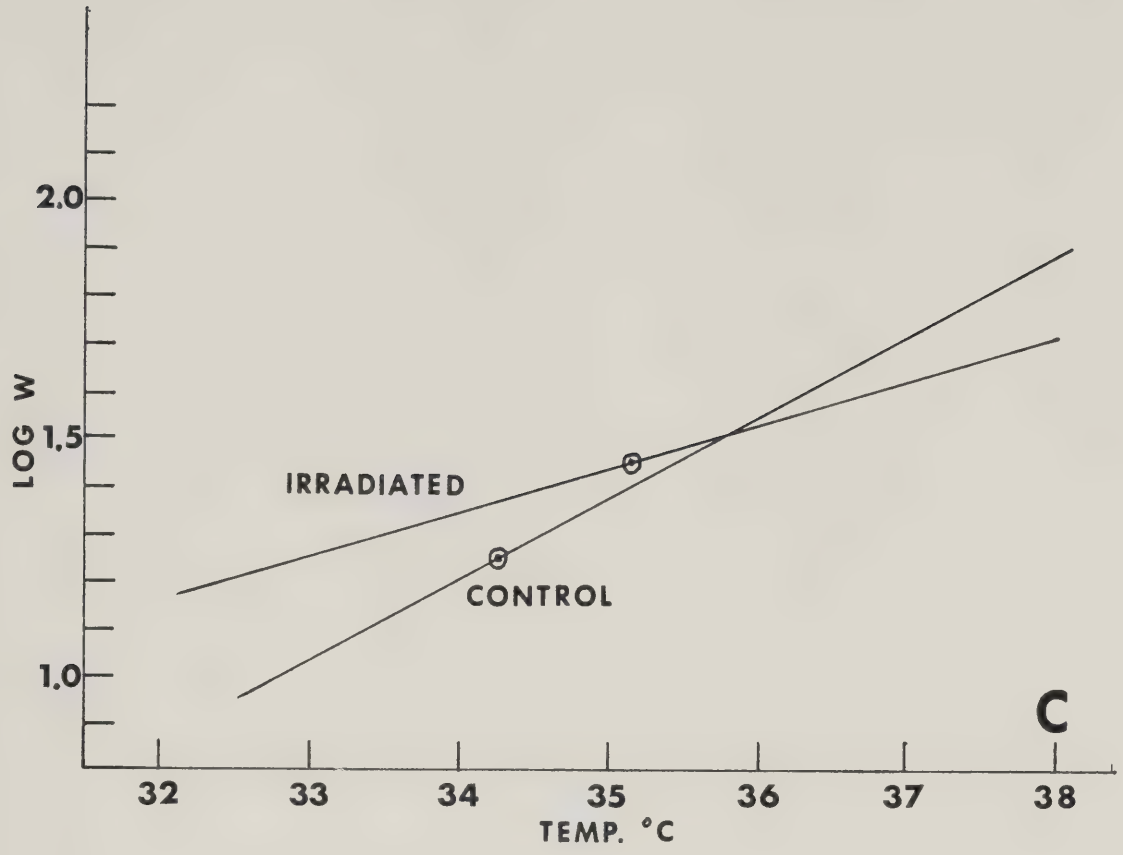
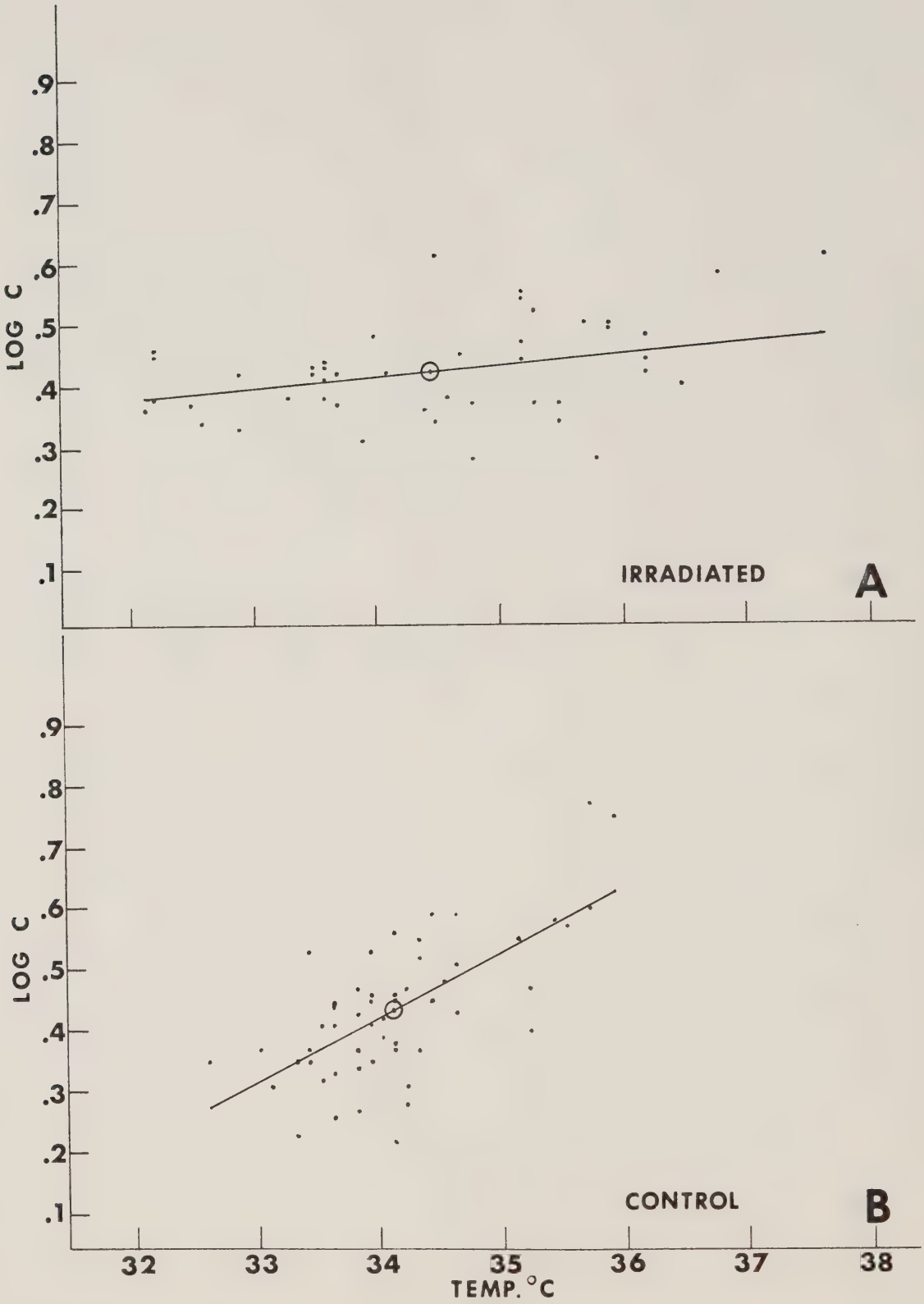


Figure 10, Graphic representations of the $\log_{10}$ transformed wet weight (log W) measurements of 4 day embryos, plotted against final embryonic body temperature. Parts A and B include scattergrams of the data and the regression lines calculated from that data for the irradiated and the control groups, respectively. These two regression lines are shown together in part C for comparison. Circled points on the regression lines are $(\bar{X}, \bar{Y})$'s.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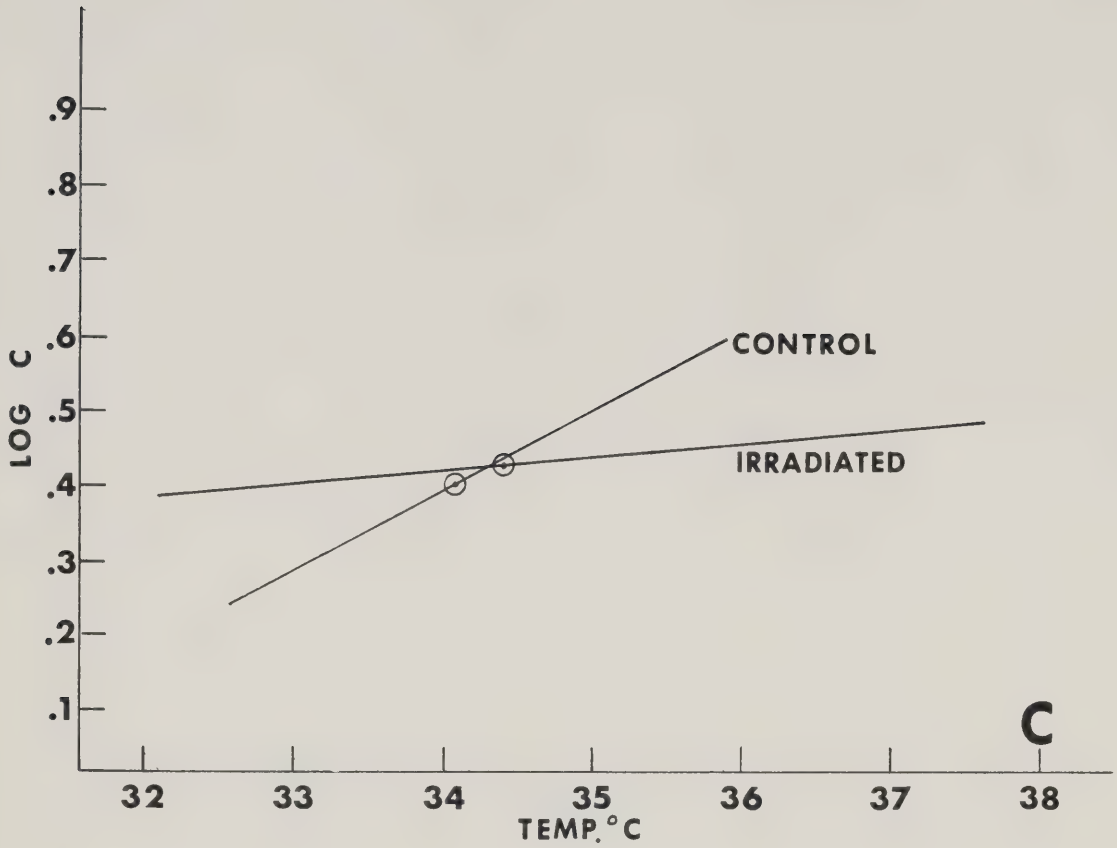
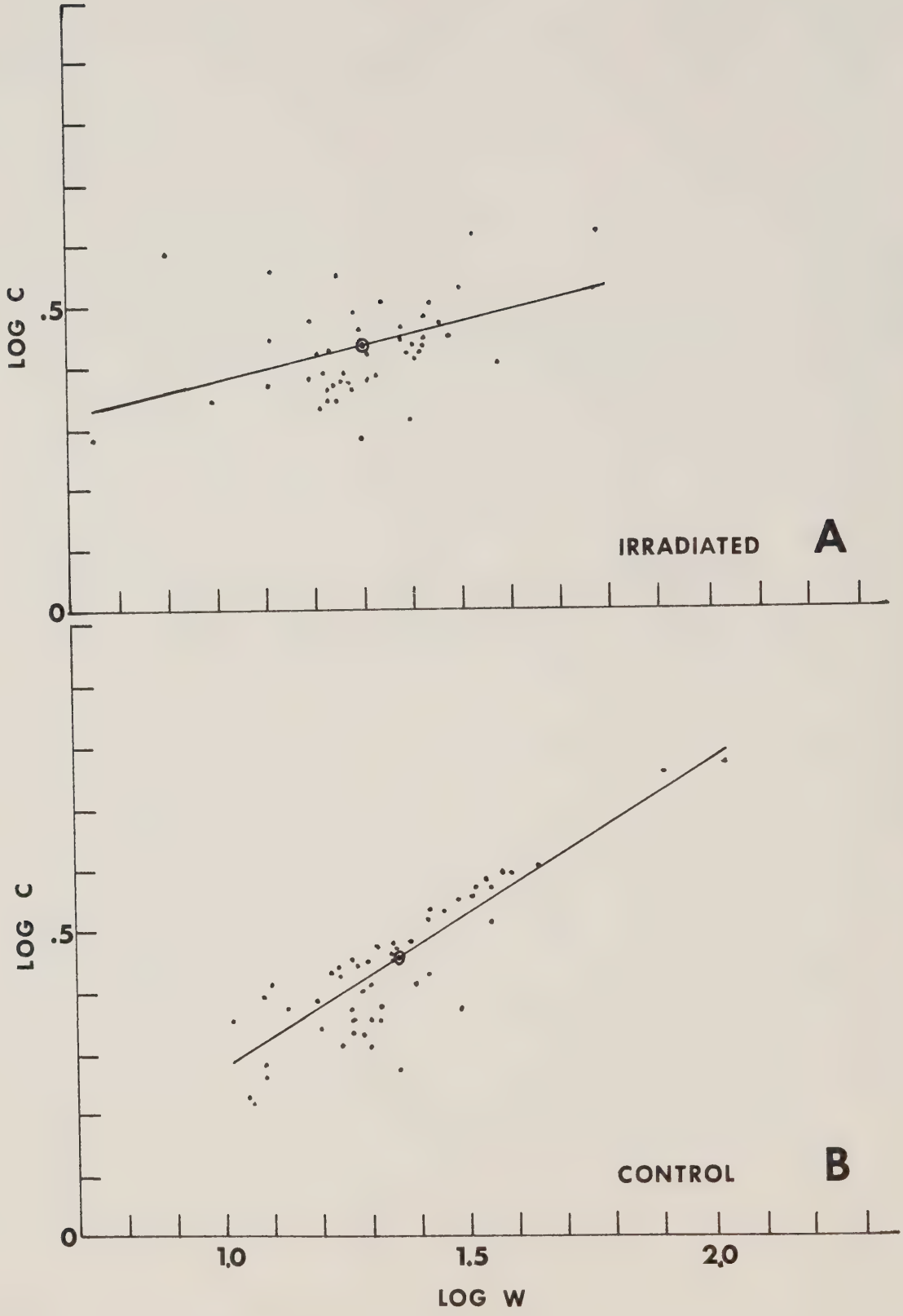


Figure 11, Graphic representations of the $\log_{10}$ transformed cranial length (log C) measurements of 4 day embryos, plotted against final embryonic body temperature. Parts A and B include scattergrams of the data and the regression lines calculated from that data for the irradiated and the control groups, respectively. These two regression lines are shown together in part C for comparison. Circled points on the regression lines are $(\bar{X}, \bar{Y})$'s.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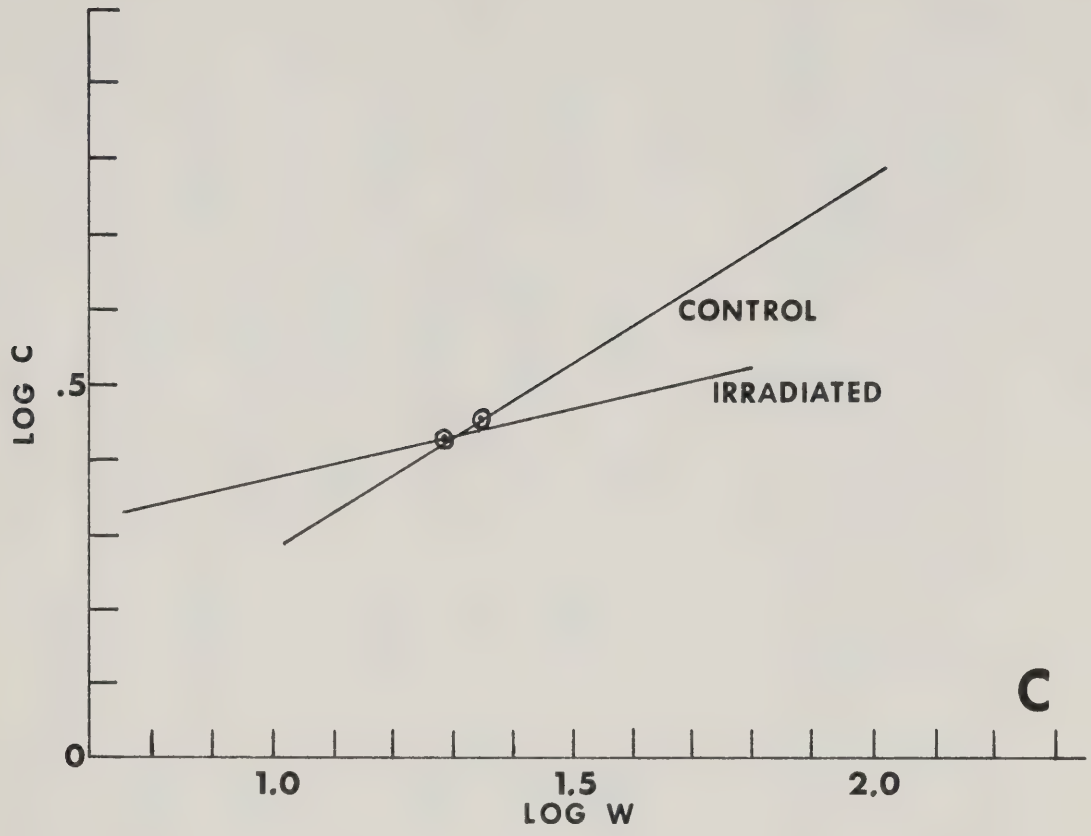
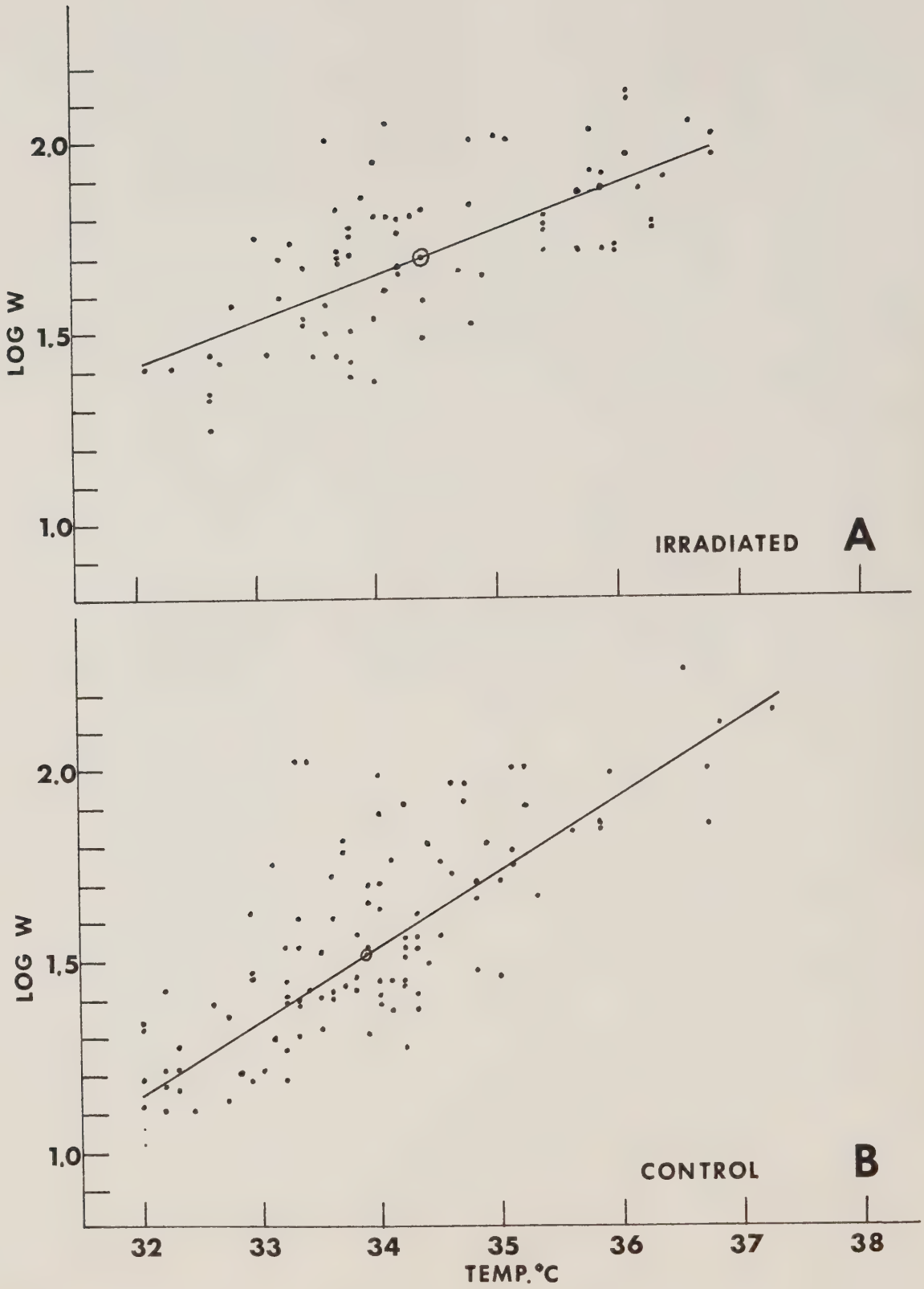


Figure 12, Graphic representations of the $\log_{10}$ transformed cranial length (log C) measurements of 4 day embryos, plotted against the $\log_{10}$ transformed wet weight (log W) measurements of the same embryos. Parts A and B include scattergrams of the data and the regression lines calculated from that data for the irradiated and the control groups, respectively. These two regression lines are shown together in part C for comparison. Circled points on the regression lines are $(\bar{X}, \bar{Y})$'s.

regression parameters	exposure regime	n	regression equation	95% CI for b'	b'_s vs b'_i	r	sig. of r	r_s vs r_i
logC vs T	sham	53	logC = 0.108T - 3.25	0.068 0.148	t=2.6052*	0.69	t=6.0104**	t=2.1637*
	irrad.	46	logC = 0.016T - 0.143	-0.002 0.032		0.38	t=2.6939**	
logW vs T	sham	136	logW = 0.144T - 3.64	0.110 0.178	t=0.0317	0.62	t=8.3034**	t=0.4620
	irrad.	110	logW = 0.095T - 1.93	0.069 0.121		0.58	t=6.8271**	
logC vs logW	sham	51	logC = 0.510logW - 0.248	0.408 0.611	t=2.6806**	0.87	t=9.2145**	t=2.6956**
	irrad.	45	logC = 0.169logW - 0.204	0.006 0.332		0.38	t=2.6624*	

Table IV, Results of the regression and correlation analysis applied to data collected from embryos incubated to 4 days, where C is the cranial length in mm, W is the wet weight in mg and T is the final body temperature in ^{o}C, of the embryo. Presented are: the regression equation, the 95% confidence limits for the regression coefficient (b'), results of the t-test for the difference between regression coefficients for the control (b'_s) and the irradiated (b'_i) embryo groups, the correlation coefficient (r), the t-test significance of r and the results of the t-test for homogeneity of correlation coefficients between control (r_s) and irradiated (r_i) embryo groups.

* - P<.05
** - P<.01


2.0
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IRRADIATED
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LOG W
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TEMP. °C

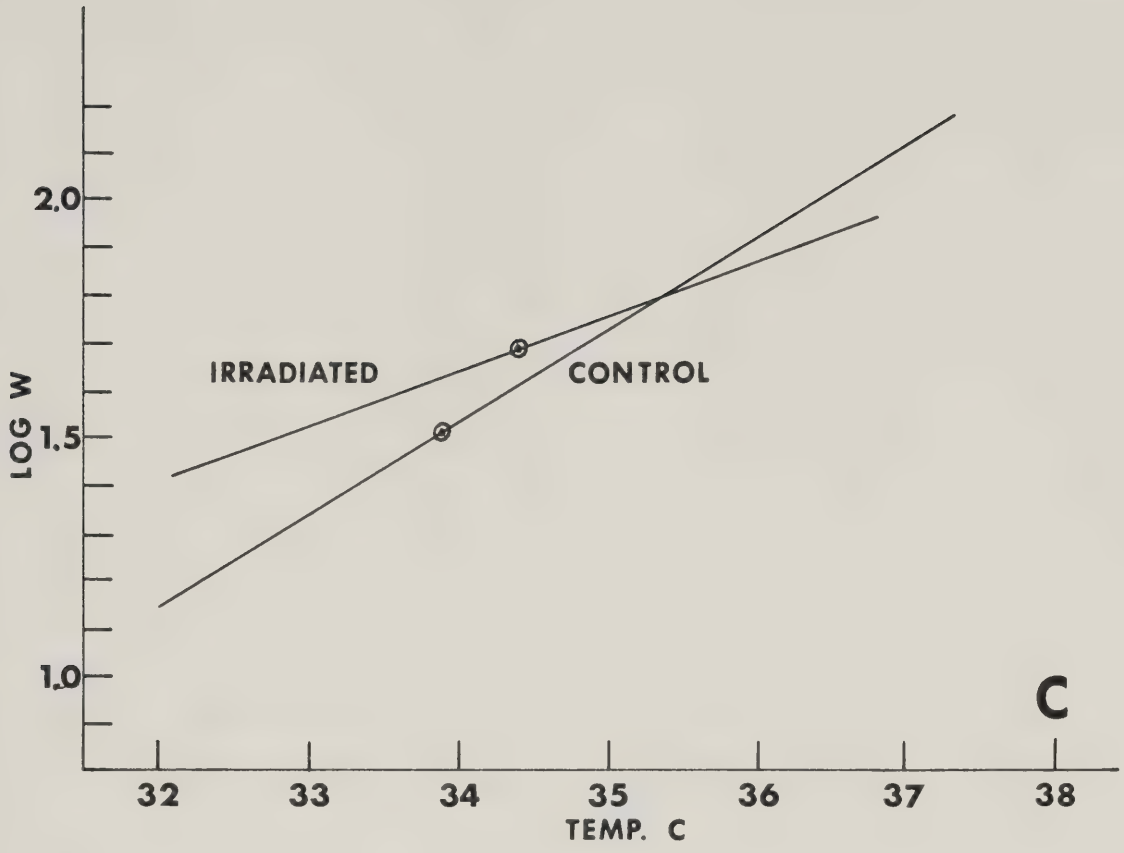
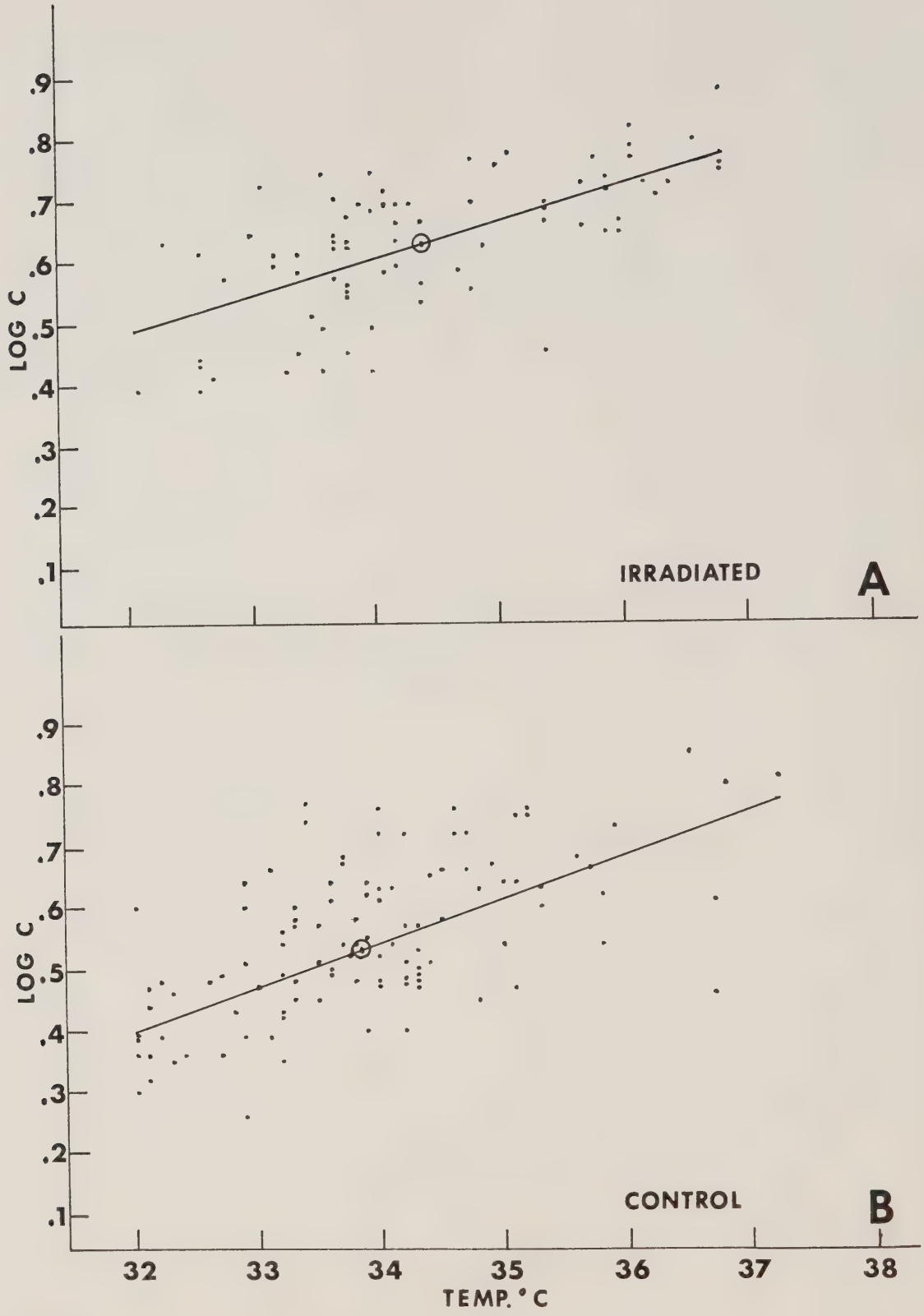


Figure 13, Graphic representations of the $\log_{10}$ transformed wet weight (log W) measurements of 5 day embryos, plotted against final embryonic body temperature. Parts A and B include scattergrams of the data and the regression lines calculated from that data for the irradiated and the control groups, respectively. These two regression lines are shown together in part C for comparison. Circled points on the regression lines are $(\bar{X}, \bar{Y})$'s.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TEMP. ° C

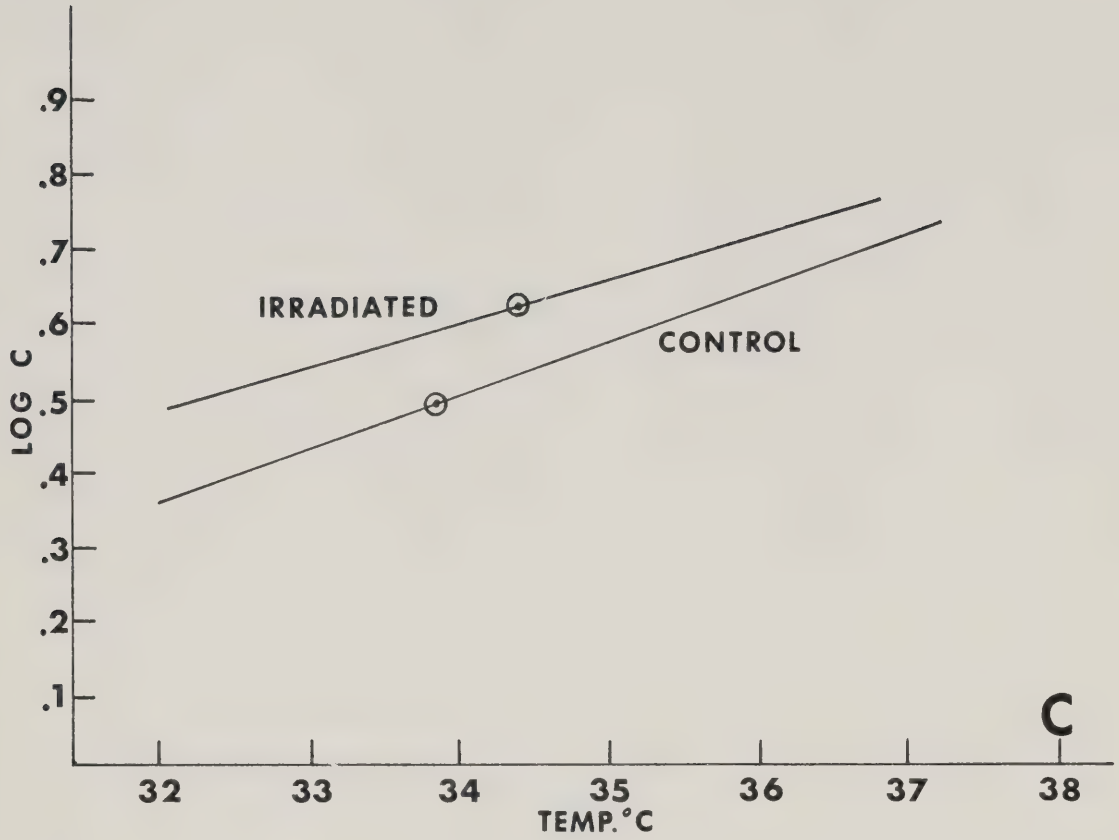
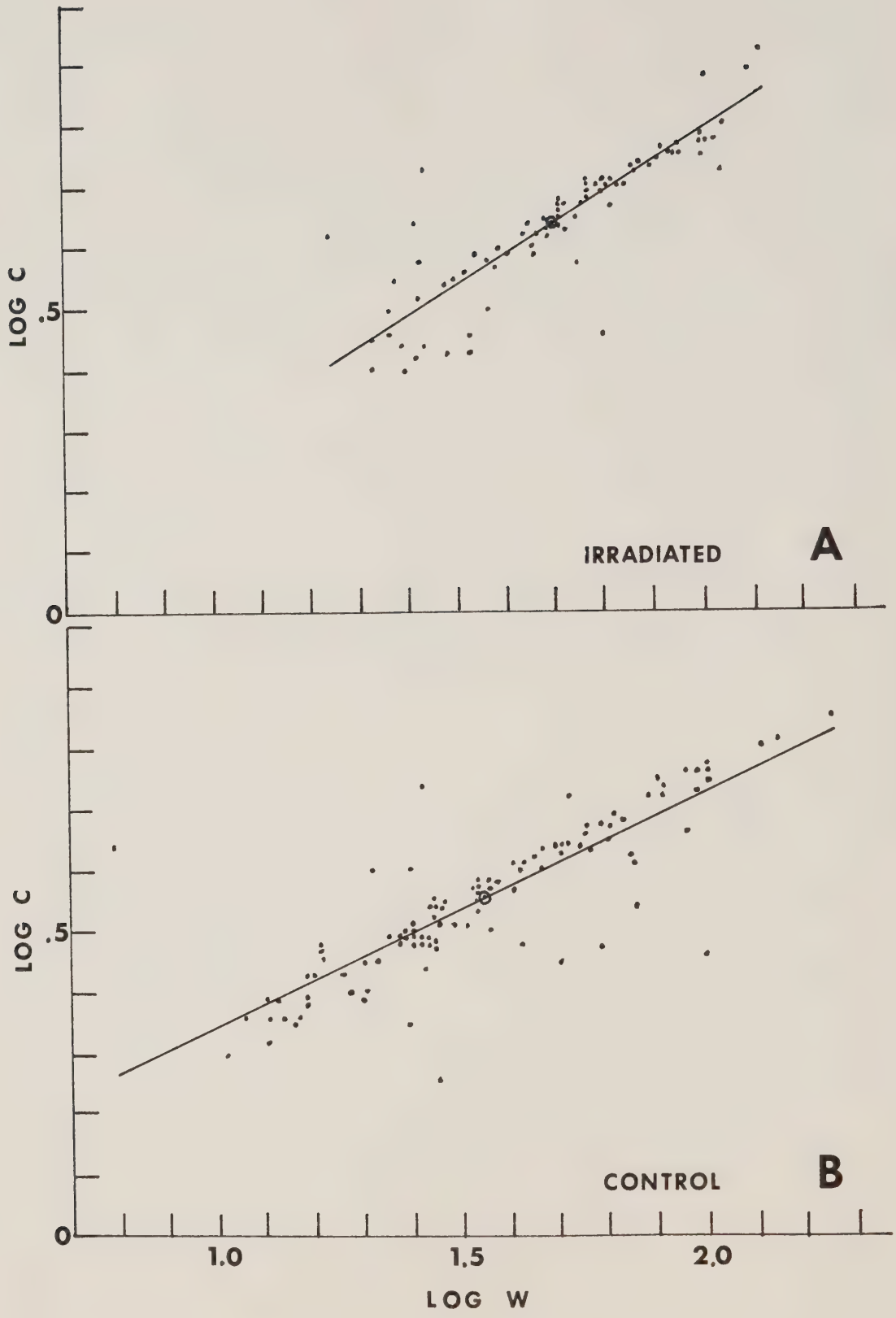


Figure 14, Graphic representations of the $\log_{10}$ transformed cranial length (log C) measurements of 5 day embryos, plotted against final embryonic body temperature. Parts A and B include scattergrams of the data and the regression lines calculated from that data for the irradiated and the control groups, respectively. These two regression lines are shown together in part C for comparison. Circled points on the regression lines are $(\bar{X}, \bar{Y})$'s.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LOG W

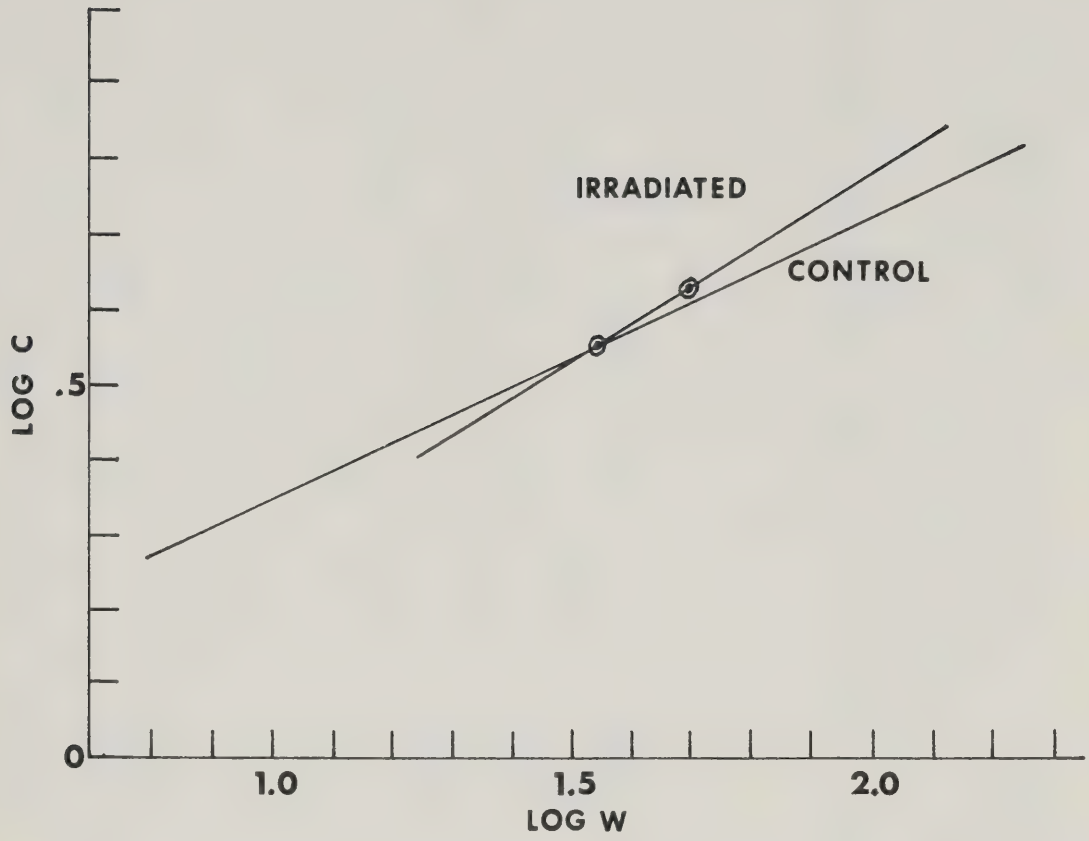

Figure 15, Graphic representations of the $\log_{10}$ transformed cranial length (log C) measurements of 5 day embryos, plotted against the $\log_{10}$ transformed wet weight (log W) measurements of the same embryos. Parts A and B include scattergrams of the data and the regression lines calculated from that data for the irradiated and the control groups, respectively. These two regression lines are shown together in part C for comparison. Circled points on the regression line are $(\bar{X}, \bar{Y})$'s.

regression parameters	exposure regime	n	regression equation	95% CI for b'	b'_s vs b'_i	r	sig. of r	r_s vs r_i
logC vs T	sham	105	logC = 0.072T - 1.87	0.052 0.091	t=0.4772	0.61	t=7.1595**	t=0.6549
	irrad.	77	logC = 0.058T - 1.38	0.041 0.076		0.67	t=6.9739**	
logW vs T	sham	109	logW = 0.194T - 5.03	0.161 0.228	t=1.5386	0.78	t=10.763**	t=1.3255
	irrad.	78	logW = 0.114T - 2.21	0.081 0.146		0.69	t=7.3439**	
logC vs logW	sham	105	logC = 0.376logW - 0.029	0.320 0.433	t=0.6586	0.82	t=11.683**	t=0.6479
	irrad.	77	logC = 0.456logW - 0.151	0.386 0.528		0.85	t=10.806**	

Table V, Results of the regression and correlation analysis applied to data collected from embryos incubated to 5 days, where C is the cranial length in mm, W is the wet weight in mg and T is the final body temperature in ^{o}C, of the embryo. Presented are: the regression equation, the 95% confidence limits for the regression coefficient (b'), results of the t-test for the difference between regression coefficients for the control (b'_s) and the irradiated (b'_i) embryo groups, the correlation coefficient (r), the t-test significance of r and the results of the t-test for homogeneity of correlation coefficients between control (r_s) and irradiated (r_i) embryo groups.

* - P<.05
** - P<.01

line was in all cases significant at the P<.01 level except for the 4 day, irradiated cranial length vs. wet weight group which was significant at P<.05 (Tables IV & V). The 95% confidence intervals for the slope, b', of the regression lines (Tables IV & V) indicate that in all but one case the slope was significantly different from zero. The lower confidence limit of the slope of the 4 day, irradiated cranial length vs. temperature group is negative while the upper limit is positive (Table IV).

The correlation of the cranial lengths of the 4 day irradiated embryos vs. temperature and vs. wet weight was not homogeneous with that of the control data. This is indicated by the significant degree of difference (P<.01) observed between the correlation coefficients of these data groups (Table IV).

Although there were no observed significant differences between the 5 day irradiated and the 5 day control embryos, the trends in the 5 day data are similar to those found in the 4 day data (Figures 10 to 15). The similarity in the wet weight vs. temperature data is, for example, revealed by the fact that in both the 4 day and the 5 day embryo groups the control regression lines are of greater slope than those calculated from irradiated embryo data. The regression lines also cross each other in both cases (Figures 10 & 13). A similar situation is observed when comparing the 4 with the 5 day cranial length vs. wet weight data (Figures 12 & 15).

CHAPTER

DISCUSSION

Significant differences were found between control embryos incubated for 4 days and those irradiated continuously with 2450 MHz microwaves during incubation for the same duration. Thermal artifacts resulting from the irradiation can probably be dismissed as a causative factor. Any measurable increases in the body temperature of the embryo caused by the microwave were included in the data analysis as a factor which would influence embryonic development. That is, any individual embryo was treated as if it had been incubated throughout at its measured, final body temperature. The final body temperature was thus viewed as the sum of the air temperature around the egg, any metabolic heat and any temperature increases resulting from the absorption of microwave energy.

The rationale for using a range of temperatures below optimal incubation temperature for chick embryos, 37.8^{o}C (Freeman and Vince, 1974) was based on the assumption that some thermal increase would probably occur as microwave energy was absorbed. The incidence of teratogenesis is known to increase progressively with deviation, in either direction, from optimal incubation temperature (Needham, 1963). In a strictly thermal sense, small amounts of microwave heating could presumably compensate for a temperature decrement in eggs incubated at suboptimal temperatures. This could decrease the

number of abnormalities expected in embryos incubated at the low temperatures used in this study. If optimal incubation temperature had been used, microwave heating could have caused an increase in the incidence of teratogenic effects. No measurable temperature increases or abnormal development attributable to microwave exposure were observed in the irradiated embryos.

Increasing the incubation temperature from sub-optimal to optimal has been reported to enhance the rate of chick embryonic growth (Needham, 1963). Assuming even that some microwave induced temperature increases in the present experiments may not have been detected, such increases, if they occurred, should probably have caused an increase in the rate of embryonic growth. However, these data show no difference in the growth rate of exposed embryos compared with that of control embryos at the same temperature (Table IV). Non-specific heating by microwaves cannot be responsible for this effect and, therefore, a mechanism not associated with physiologically significant thermal changes must be considered. As indicated previously, thermal changes always accompany the absorption of microwave energy. In this case the thermal changes were insufficient to elicit a measurable physiological response (ie. in growth rate), where one would have been expected. If there had been a temperature increase large enough to cause a change in the growth rate, the other effects observed might have been masked.

The observation that no teratogenic effects resulted from exposure to microwaves is not in agreement with observations made by Van Ummerson (1961). She used a high intensity (200-400 mW/cm^2)

short duration (1-15 min.) microwave exposure regime. The abnormalities Van Ummerson reported may therefore have been the result of microwave heating. In her experiments the temperature of the yolk 4 mm below the embryo reached as high as 59°C, measured by an implanted thermistor.

Van Ummerson (1961) pointed out that X-ray radiation, while causing no appreciable heating, induced teratologic effects similar to those caused by heating with microwaves. On this basis she suggested "... the possibility of a non-thermal influence exerted by microwave radiation which could be acting either concomitantly or synergistically with the obvious thermal effects of the radiation..." This possibility cannot be denied. The analogy between X-ray and microwave effects is, however, limited since their quantal energies are so different. The ionization potentials for biomolecules are generally taken to be of the order of 10 eV (Cleary, 1973) while the quantal energies of microwaves and X-rays are 10^{-3} to 10^{-6} eV and approximately 1 KeV, respectively (Weidner and Sells, 1973). X-rays can therefore directly cause the ionization of nucleic acids and proteins, which could adversely affect development. The intensity of microwaves necessary to exert a comparable effect would thermally destroy any biological system in seconds (Cleary, 1973).

The results presented here do, however, support the idea that there may be some interaction between the effects of temperature and the effects of microwaves on the growth and development of chick embryos. The cranial lengths of embryos incubated for 4 days varied with temperature and with wet weight in a significantly different

manner for irradiated than for control embryos. The magnitude and the direction of the cranial length effect also appears to be temperature dependent (Fig. 11).

The finding that microwaves have a significant effect on the cranial lengths of 4 day embryos and that there were insignificant differences between the wet weights of irradiated and control embryos suggests that microwaves affect embryonic development. The implication of a developmental effect, from the data on the embryonic growth parameters used here, is supported by the results of Demorest (1978). He observed a high degree of correlation between wet weight, cranial length and developmental stage (Hamilton-Hamburger) of the chick embryo. Similar significant relationships were observed between the cranial lengths and wet weights of control embryos in the present experiments but not in the irradiated 4 day embryos.

In the cases when regression coefficients of the irradiated embryo groups were significantly different from those of the controls (ie. 4 day cranial length vs. temperature and 4 day cranial length vs. wet weight)(Table IV), the correlation coefficients between groups were also significantly different. This observation supports the suggestion made above: that microwaves may have affected embryonic development in these experiments. However, another possibility is that this inhomogeneity among correlation coefficients may also be due to the fact that the field pattern incident to the eggs was not uniform (Fig. 7, Table 1). Consequently the proposed microwave effect on the cranial length of chick embryos may be

viewed as either intensity or dose rate dependent.

The cranial length effect recorded here appears to be a genuine microwave bioeffect, possibly non-thermal. The fact that no similar effect was observed in 5 day irradiated embryos is difficult to understand. It may be that microwaves affect the cranial length of chick embryos only at a certain stage (or stages) of embryonic development. However, the embryos of this study were not all growing at the same rate. Although the body temperature of an individual embryo varied in these experiments by ± 0.4°C with time, the temperature of different embryos varied between 32° and 38°C and, therefore, these were growing at different rates. Thus, the embryos would have reached different stages of development during the same period of incubation. If the cranial length effect is stage-specific, two different irradiated embryos developing under different temperature regimes might reach a microwave-susceptible stage at different times relative to the onset of incubation. At 4 days of incubation perhaps only half of the irradiated embryos, those with higher body temperatures and thus more developed, had been affected by the microwaves. This could account for the differences in the regression coefficients (ie. the slopes) of control and irradiated 4 day embryo groups. There were no significant differences between the regression coefficients of control vs. irradiated 5 day embryos. One explanation might be that by 5 days of incubation all the embryos had developed to a stage beyond the proposed microwave-susceptible one and that the difference exhibited by 4 day embryos had disappeared. By 5 days

the irradiated embryos would only differ by reason of incubation temperature just as did the control embryos.

Another instance of a stage specific reaction to microwaves has been identified by Chernovetz (1978). She reported that the effect microwaves have on the fetal mass of rats is dependent on the stage of development of the fetus at the time of exposure. The direction and the magnitude of the mass effect observed by Chernovetz depended as well on the dose rate of the exposure.

The mechanism for the microwave bioeffects observed in these experiments cannot be determined from the present results. The following, describes some of the possible mechanisms for the interaction of microwave radiation with biological membranes, any of which may be responsible for the above microwave bioeffects.

MacGregor (1970) has calculated that a human head exposed to a 10 mW/cm^2, 1 GHz field would develop an intracranial potential gradient of approximately 200 V/m. Of the resulting 36 mA/cm^2 current about one half, or 18 mA/cm^2, would be expected to pass through neuronal membranes. This would, theoretically, cause a decrease of approximately 0.2 mV in the resting transmembrane potential (MacGregor, 1970). Schwan (1971) suggested, however, that such a reduction could not significantly influence the action potential amplitude or conduction velocity of single neurons. MacGregor (1970) and Frey (1971, 1974), while supporting this conclusion, have argued that such a drop in transmembrane potential, although very small, could affect higher nervous function: the increased reflex response in cats exposed to 10 mW/cm^2, 2430 MHz radiation as

observed by McRee (1976), for example. The rationale for their argument is that apparently insignificant changes in transmembrane potential could have a significant neurological effect after these changes are integrated, via temporal and spatial summation, through the increasing functional levels of the central nervous system (Frey, 1971). This could explain the above reflex effect as well as the electroencephalographic desynchronization reported by Baranski and Edelwejn (1974), the subjective disorders such as insomnia and loss of memory described by Presman (1970), and the observed fluctuations in neuroelectric potentials of the cat brain stem (Frey, 1967).

This theory that microwaves decrease membrane potential should, if supported, apply to all biological tissues and not only to nervous tissue. In the light of Cone's recent work (1973, 1974, 1976) in which he reported a high degree of correlation between transmembrane potential and mitotic index, it follows that microwaves could in this way also affect the growth and development of chick embryos. Again, a small change in the transmembrane potential of a single cell would probably be insignificant but when the possible growth effect is summed over a whole population of rapidly dividing cells, the effect may become measurable.

Cone (1976) has reported that even fully differentiated neurons may be stimulated to divide by depolarization of their membranes with ouabain, an inhibitor of Na^+/K^+ ATPase. Accompnaying the 80% decrease in membrane potential, Cone (1973) observed a 3.3-fold increase in intracellular Na^+, a concomitant decrease in intracellular K^+, and an increase in RNA synthesis followed by an increase in DNA

synthesis. Cone (1974) suggests that increased intracellular concentrations of Na^+ may be indirectly responsible for the initiation of the mitotic process, for instance by Na^+ involvement in the genetic activation or repression processes. The possibilty that microwaves may alter growth rate via this mechanism is supported by Baranski's (1974) report that low level microwaves increase red blood cell membrane permeability to Na^+ and K^+.

Although microwave-induced changes in membrane potential would directly alter the Na^+/K^+ ratio within the cell, the same effect could result from a change in the function of the Na^+/K^+ transport system. Cone (1974) postulates that the activity of membrane bound enzymes such as Na^+/K^+ ATPase may be dependent on the electric field of the polarized membrane. A change in the Na^+/K^+ ratio within the cell may, therefore, be an indirect effect mediated by the influence of microwave-induced changes in membrane potential on membrane-bound enzymes. Alternatively, microwaves may act directly on membrane bound molecules such as Na^+/K^+ ATPase. There is some experimental evidence which indicates that an externally applied electric field, such as that which could be generated in tissues exposed to microwaves, could potentially alter the steric configuration of one of these membrane-bound enzymes or receptor molecules, such that its function would be qualitatively and/or quantitatively changed. Servantie et al (1974) have reported decreased sensitivity to curare-like drugs in rats exposed to microwave radition, suggesting a decrease in the binding energy of the drug to acetylcholine receptors. This finding seems to agree with Nikogosyan's (1960, 1964; cited in Presman, 1970) observation of reduced cholinesterase activity in

neural tissue of microwave-treated animals.

The proposal that microwaves may directly affect membrane bound molecules is supported by Frohlich (1975). He pointed out that transmembrane potentials, while small in absolute terms, amount to an overall electric field intensity of 10^7 V/m within the membrane. Such a field could conceivably lead to a change in the electrical symmetry, and thus probably in the spatial symmetry, of a membrane-bound molecule. As opposed to the configuration of an enzyme in solution, the same enzyme, membrane-bound and subjected to an electric field, may take on one of it's metastable configurations as the favoured state (Frohlich, 1975). Further to this, Frohlich (1975) has suggested the possibility of frequency specific effects in biological systems exposed to mm. wavelength electromagnetic radiation. Although the relaxation frequency of most enzymes in solution has been found to be about 3 KHz (Yeargers, 1975), the relaxation frequencies of membrane-bound enzymes have been calculated, on a theoretical basis, to be in the GHz (mm.) range (Frohlich, 1975). Such a shift in the dielectric behavior of membrane-bound molecules has also been proposed by Devyatkov (1975), and has been suggested as biologically relevant by Grundler et al (1977). Grundler demonstrated a series of frequency specific growth rate effects in yeast cells exposed to low intensity GHz microwaves. By extension, this may implicate a non-thermal, frequency specific, probably molecular mechanism for the effect on chick embryo development described herein.

Changes in the electrical symmetry of a single enzyme which might be caused by microwave radiation of low intensity would be minute (200 V/m induced by 10 mW/cm^2, 1 GHz microwaves as opposed to a 10^7 V/m gradient within a membrane). Any functional changes at the single cell level would, consequenctly, also be quite small. One must visualize that this effect is somehow integrated or summed in order to establish, at least theoretically, what these changes imply for the whole organism or tissue. The possibility cannot be denied that this may be one of the mechanisms by which microwave bioeffects, including the developmental effect described here, occur.

The configurational shifts which may be brought on by potential changes in the electrical symmetry of an enzyme due to changes in a membranal electric field must be understood in terms of membrane molecular composition. The membranes of different tissues have been shown to be quite diverse in composition (Lehninger, 1970). Therefore, while the reaction of different enzymes in the same membrane to the same microwave frequency would certainly be expected to be variable, the reaction of the same enzyme exposed to a constant frequency could also be different in different tissues. This may account for the microwave-induced change in the cranial length to wet weight ratio of 4 day embryos. The microwaves might have affected the growth rates of several parts of the embryo to different degrees at different times for the above reasons.

With respect to the above suggestion that microwaves affect growth through a change in the Na^+/K^+ balance inside the cell, three mechanisms are proposed: 1) a direct effect on the membrane potential influencing the passive movement of ions, 2) microwave

induced changes in the electric field within a membrane secondarily affecting enzmes responsible for active ion transport and 3) a direct effect on membrane-bound molecules such as Na^+/K^+ ATPase. Further to the fact that any of these would change the intracellular Na^+/K^+ ratio, all would also be temperature sensitive. Passive as well as active ion transport is dependent on, among other parameters, membrane fluidity which in turn is temperature sensitive (Fox, 1973). With respect to the proposed enzyme effects, the influence of temperature induced changes in membrane fluidity affecting the enzyme would be in addition to any slight changes in enzyme kinetics resulting directly from temperature changes. This mechanism could help to explain the temperature sensitivity of the effects seen here in developing chick embryos. This would apply whether changes in the dielectric behavior of membrane-bound molecules are due to the direct action of microwaves on those molecules or occur secondarily via the action of microwaves on transmembrane potential, as proposed by MacGregor (1970).

The preceeding is highly speculative and is not a complete list of the possible mechanisms of interaction of microwaves with biological systems. Those discussed, however, appear to be likely both in biological and physical terms. This hypothesis, that microwaves affect biological membranes, could be tested using artificial bilayer lipid membranes as an experimental system. Any microwave effect or absence of effect on the passive and/or active transport of ions across such membranes may provide valuable information needed to elucidate the mechanism of action of microwaves in living systems.

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